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ON THE METABOLISM OF BACTERIA*

C. H. WERKMAN AND H. G. WOOD

Iowa Agricultural Experiment Station and Industrial Science Research Institute, Iowa State College, Ames, Iowa

Leeuwenhoek and His Animalcules

It remained for a Dutch lens-grinder, in the quaint old city of Delft, to see for the first time and accurately describe bacteria, probably in the year 1675. Anthony van Leeuwenhoek, the father of microbiology, transmitted his findings to the Royal Society of Great Britain in a series of 112 communications dealing with various scientific topics. His wide range of microscopical discovery was a product of his shrewd curiosity and diligent persistence which 1 d to preeminence among his contemporaries. He ground his own lenses—better ones than had been ground before—and today we know that they reached close to the perfection of simple lenses. Leeuwenhoek turned his lenses to the investigation of a wide variety of substances but his exact microscopic technique, which Leeuwenhoek did not choose to reveal, remains a matter of conjecture. In his 5th observation on pepper-water, entry of August 6, 1676, occ   s Leeuwenhoek's famous remark: "My method for seeing the v y smallest animalcules and minute eels, I do not impart to others; nor how to see very many animalcules at one time. That I keep for myself alone" (*cf.* Cohen, 1937, for translation of Leeuwenhoek's letter).

Students of microscopy, faced with Leeuwenhoek's unwillingness to reveal his methods—and there is no question that Leeuwenhoek actually saw bacteria—have puzzled for many years to understand how bacteria were made visible by the relatively primitive optical systems at his disposal. Leeuwenhoek worked with only simple lenses; they were good lenses, but so far as evidence shows, without the aid of condensers or diaphragms. It is not unlikely that Leeuwenhoek had discovered the method of dark ground illumination. Dobell (1932) declares his conviction that the method must have involved dark ground illumination, and in support quotes a remark by Leeuwenhoek to the effect that red blood cells were sharp and clean like sand-grains on black taffeta.

SYSTEMATIC POSITION OF BACTERIA

Bacteria were considered to be animalcules by Leeuwenhoek; Cohn in 1854 concluded that bacteria were plants. Today bacteria are recognized as unicellular, microscopic plants, multiplying by binary fission. Bacteria range from rather well defined plant forms,

on the one hand, to those showing definite animal characters on the other. No sharp lines of demarcation exist in Nature. Morphological and physiological characteristics change gradually; they are *continuous* functions. Thus Nature blends from type to type with no particular barriers; man for his own convenience attempts to visualize Nature with definite lines of demarcation. In figure 1 is a schematic arrangement showing the relative position of the bacteria with reference to the plant and animal kingdoms.

The more plant-like nature of bacteria in general is attested by both morphological and physiological characters. Among the more convincing arguments, should be mentioned, first their relative ability to synthesize in large measure, the substances of a vitamin nature required for growth, multiplication, and provision of their energy needs. Among the bacteria, and those forms showing relatively close relationship to groups of the Thallophyta, *i.e.*, the fungi and algae, synthesis of vitamins, coenzymes and similar factors essential in the assimilative and dissimilative processes, occurs quite generally. Such syntheses have not been reported among the protozoa. Thus the plants are characterized by their broader synthetic properties; animals are notably deficient in this respect.

Secondly, the marked ability to assimilate CO_2 may serve to differentiate many of the bacteria as plants. A large number of bacteria are able to synthesize their own carbon compounds, *i.e.*, autotrophic forms. The autotrophic bacteria grow in inorganic media. The purple and green forms of the Thiobacteriales utilize CO_2 in a truly photosynthetic manner; they contain bacteriochlorophyll; others are chemosynthetic. The latter group obtain their energy not from the sun's rays but from the oxidation of their substrate. All true autotrophs synthesize their carbon-containing constituents from carbon dioxide.

It has been generally accepted that the oligocarbophilous bacteria belong to the autotrophic group; in fact, the term oligocarbophilous applied to a microorganism, implied that it was an autotroph until recent work established the rather general fixation of carbon dioxide by organisms in representative genera of the heterotrophs, such as *Propionibacterium* and *Escherichia.*

Thirdly, indirect proof of the plant nature of the bacteria lies in their close relationship to groups having more pronounced plant characteristics, *i.e.*, Cyanophyceae and fungi. In this connection,

however, it is to be pointed out that the Rickettsiales and Spirochaetales show a relationship to the protozoa. The nature of the viruses seems to place them in the zone between plants and animals, representing undifferentiated protoplasm. Character of cell wall and type of reproduction have also been used to differentiate bacteria as plants. It is to be mentioned, indeed, that differentiation of organisms into plants and animals is, after all, for purposes of expediency and reference.
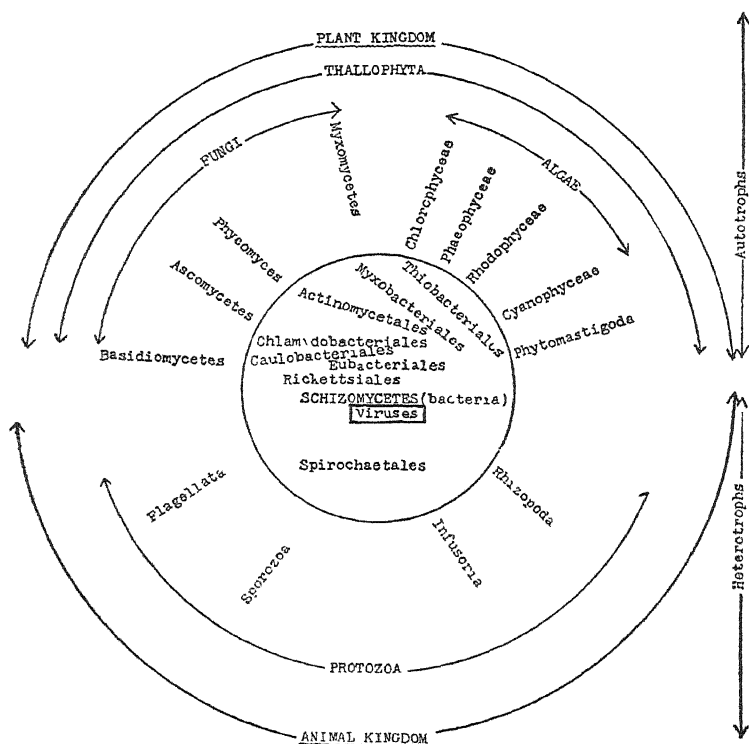
Systematically, bacteria are recognized as constituting a class, Schizomycetes. The Eubacteriales or "true bacteria" comprise the typical and better known forms and will furnish the basis for the greater part of this discussion. Found within this order are the commonly known bacteria, such as *Bacillus subtilis,* the hay bacillus; *Escherichia coli,* common inhabitant of the intestine; *Aerobacter aerogenes,* found on grain; and the well known lactic acid-forming

bacteria, *Lactobacillus bulgaricus* and *L. acidophilus*. Many of the pathogens are also found in this order.

The Eubacteriales are related to the algae through the Chlamydo-bacteriales, the Caulobacteriales and the Thiobacteriales. The Caulobacteriales are non-filamentous rod or spherical bacteria growing characteristically upon stalks formed by a secretion from one end or side of the organism. The Chlamydobacteriales are filamentous, and are commonly known as the iron bacteria. Some are obligate autotrophs, *e.g., Didymohelix ferruginea;* others are facultative, *e.g., Leptothrix ochracea.* Winogradsky (1888*a*) apparently first recognized the iron bacteria as a group. They find practical significance in causing clogged water pipes, discolored and off-taste water. The Actinomycetales are frequently filamentous, branching, develop conidia, and have mold-like colonies.

Among the Thiobacteriales or sulfur bacteria are those which contain bacteriochlorophyll and bring about the oxidation of hydrogen sulfide, sulfur oxides and even organic substances. Their cells show a close relationship to the blue green algae.

The Myxobacteriales are slime mold-like with motile rods aggregating to form pseudo-plasmodia and may later produce fruiting bodies on aerial stalks.

The Spirochaetales are generally considered protozoan-like, having spiral, flexuous cells.

ADAPTIVE BEHAVIOR OF BACTERIA

Bacteria may be considered as colloidally dispersed protein sponges filled with enzymes. Each acts as a relatively independent individual in which an astounding harmony exists in the behavior of a myriad of enzymes. We know comparatively little regarding the formation, occurrence or behavior of the enzymes in the living cell.

Wortmann (1882) observed that an unidentified bacterial cell formed amylase when grown in media containing starch, whereas no amylase was formed in a starch-free medium. On the other hand, yeast always produced invertase regardless of the presence of sucrose in the medium. Since then the influence of environment on the formation of bacterial enzymes has been studied by a relatively large number of investigators. It is convenient at present to recognize the existence of two types of adaptation—environmentally im-

pressed and genetic. Specific recognition of each and every change may not be readily accomplished. Genetic adaptation involves a gene change and the new mutant may prove relatively stable. The mutant under favorable conditions will crowd out the old type and will persist. Environmentally impressed adaptation results from the presence of a specific substrate; the characteristic reaches its maximum manifestation in the immediate culture to which the specific substrate has been added and ceases to exist on removal of that substrate. Karström (1930, 1938) designated as adaptive those enzymes formed as a specific response to the presence of a substrate, and differentiated them from constitutive enzymes which are always produced by a cell, regardless of the substrate. Karström's terminology is convenient and has found wide usage, although, as pointed out by Dubos (1940), it does not adequately describe the complex influence of environment on the enzymatic constitution of the bacterial cells. Thus, alanine does not increase the formation of its deaminase in *Escherichia coli* and the presence of glucose may all but eliminate it (Stephenson and Gale, 1937). Again, *Proteus vulgaris,* grown in *l*-leucine or *d*-isoleucine media, is rich in urease, whereas when grown in Uschinsky's synthetic medium only traces are formed (Jacoby, 1917; Penfold, 1911). The formation of an enzyme may, therefore, be stimulated by a substance unrelated to its substrate (*cf*. Quastel, 1936, 1937).

It is to be recognized that the environment may be responsible not only for the production of adaptive enzymes but may induce genetic changes or provide the necessary conditions for the selective development of a mutant to predominance. Mutation is constantly occurring in bacterial cultures, probably at a much greater rate than generally suspected; however, owing to an environment favorable to the parent organism, the mutant is suppressed. If grown in a medium more favorable to it than to the parent, the mutant will grow and dominate.

It is probably not necessary for bacteria to reproduce in order to develop new enzymes. Stephenson and Stickland, working with hydrogenlyase (Stephenson, 1937; Stephenson and Stickland, 1932, 1933) and Stephenson and Yudkin (1936) with galactozymase, are of this opinion. They demonstrated enzyme formation within one hour in a washed cell suspension plus substrate. Little is known as to the mechanism of adaptive enzyme formation. The substrate

may furnish a chemical structure essential to the synthesis of the enzyme. Yudkin (1938) formulated a mass action theory in which he assumed that a new enzyme is not formed but only an increase in amount of one already present in traces. The ability of bacteria to ferment synthetic organic compounds not occurring in Nature would seem to be an uncanny foresight on the part of the organisms to elaborate, even though in traces, an enzyme to attack a compound never before contacted. According to Yudkin, the enzyme is in equilibrium with a precursor, and union with the substrate, thus removing the enzyme from the equilibrium, results in increased formation. It would seem that a study of specific enzyme adaptation should throw light on the problem of biological adaptation.

Bacteria show marked ability to adjust themselves to an environment by both genetic and environmentally impressed variation. Therefore, the dissimilation of carbohydrates can no longer play as important a rôle as in the past in the differentiation of species or genera without certain safeguards. Among these are: first, the past cultural history of the unknown microorganism must be known; secondly, the culture must be grown, previous to identification, in suitable standard media and under standard and uniform conditions. The whole technique of determining fermentation reactions in the identification of bacteria needs reinvestigation.

Wiggert and Werkman (1939) have shown that two distinct types of cells of *Propionibacterium pentosaceum* develop with respect to their ability to dissimilate phosphoglyceric acid, a cardinal intermediate in cellular dissimilation of carbohydrates. When the organisms are grown in the presence of sodium fluoride, known to prevent the fermentation of phosphoglyceric acid, cell suspensions of *P. pentosaceum* fail to attack the acid (no fluoride present) but will dissimilate glucose, although apparently not by way of the Meyerhof mechanism which involves phosphoglyceric acid as an intermediate. When the bacteria are grown in the absence of sodium fluoride, the cells apparently attack glucose, dissimilating it (as well as phosphoglyceric acid) by way of phosphoglyceric acid.

Bacterial variations must be differentiated from effects due to differences in the physical or chemical environment during culturing, since the presence of mere traces of substances may provide luxuriant growth or activity.

With regard to the existence of a form of sexual reproduction

among bacteria, relatively little can be said with assurance. Certain it is that bacteriologists are again reconsidering the evidence, and it is possible that a modified form of sexual reproduction may be found to exist among bacteria—particularly among certain forms. There is no adequate proof, in the light of our present knowledge, for or against sexual reproduction. It is desirable, however, to reinvestigate the whole problem, using modern methods.

NUTRITION AND GROWTH

Adsorption, membrane equilibrium, diffusion, permeability and surface reaction play essential rôles in providing and maintaining a suitable chemical environment for the nutrition of bacteria, to provide the energy essential to life. The complex of reactions results in biological oxidation in which the available energy is used in building cell substances and in carrying on various types of activity; in fact, energy is the underlying cause of all change. Continuous change within a cell is a manifestation of life; this may be expressed as a constant attempt of a cell to reach a "fixed equilibrium" or stable state of all of its occurring reactions. The chemical nature of biological oxidation will be discussed later; at this time only those aspects generally classed under nutrition will be discussed, *i.e.*, the processes by which growth is promoted. The term is not as inclusive as metabolism, and will not involve a discussion of the intermediary metabolism.

The food requirements of bacteria probably differ more profoundly from species to species than in any other class of organisms. It is convenient to visualize a spectrum of the nutritional requirements of bacteria. Bacteria which obtain their nutrients solely from inorganic compounds may be placed on the left (Fig. 2). They are known as autotrophs (capable of self nourishment). Autotrophs synthesize all of their carbon compounds from carbon dioxide (or carbonates); their nitrogen requirements are met by inorganic ammonium salts, nitrates or nitrites. Thus by definition an autotroph is able to multiply in an inorganic medium. It must have a source of carbon in the form of carbon dioxide or carbonates, of nitrogen, usually ammonium salts, and of energy. The fulfilment of the energy requirement is of interest inasmuch as many of the known chemosynthetic autotrophs use oxygen as a hydrogen acceptor, a process known to yield a maximum of energy, as compared with anoxybiontic processes.

Autotrophism is extended to all required elements, *e.g.*, sulphur, phosphorus, magnesium, manganese and iron. The essential point of autotrophism is that the organisms obtain their energy and build their cellular components and essential factors of the nature of vitamins from inorganic nutrients. An autotroph which reduces CO_2 by chemical energy to form its cellular carbon compounds is chemosynthetic in contrast to those which assimilate CO_2 by the aid of radiant energy, *i.e.*, photosynthesis. The basic similarities of the two processes will be discussed later.

NUTRIENT REQUIREMENTS OF BACTERIA

Bacteria

Autotrophs
Synthesize all cell constituents from inorganic materials, *e.g.*, CO_2, NH_3, and inorganic ions

Heterotrophs
Require organic carbon

Chemosynthetic
Utilize chemical energy

Photosynthetic
Utilize radiant energy

Saprophytic
Do not require living tissue

Parasitic
Require living tissue
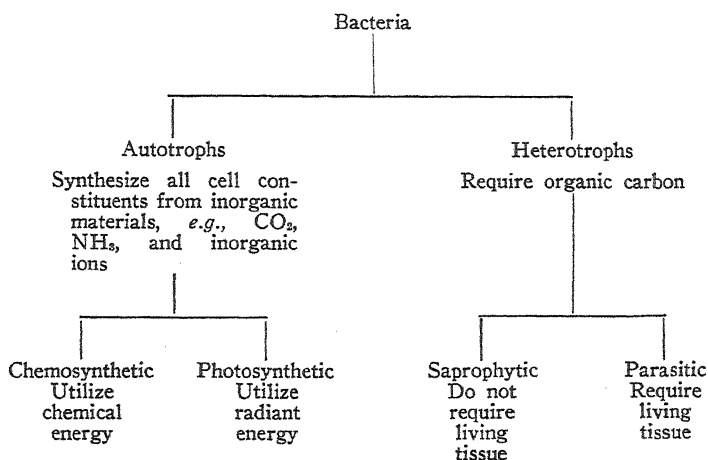
FIG. 2

On the other extreme of the spectrum are the heterotrophic bacteria which require complex sources of carbon, such as glucose, and sometimes of nitrogen, such as peptone, or even constituents closely associated with the living cell. This spectrum of bacterial nutrition portrays, in fact, the range of bacterial differentiation from the anaerobic autotroph to the extremely parasitic Rickettsiales requiring living tissue.

The heterotrophs may be separated for convenience into two groups, the saprophytes which utilize dead organic matter, and the parasites which can be cultivated only in the presence of specific living tissue. Here again, there are many intergrading forms. Between the autotrophs and the heterotrophs we also find many intermediate forms; thus the propionic acid bacteria utilize CO_2

although they are classed as heterotrophs requiring complex sources of carbon and flourish best when provided with the complex substances of yeast. It is for this reason that a *spectrum* of bacterial nutrition has been pictured rather than a rigid classification into sharply demarcated groups.

It is desirable, however, to discuss further the changes exhibited by organisms as we proceed from the obligate autotrophs to the highly differentiated heterotrophs. The nutritional requirements of the autotrophs are relatively simple in comparison, a condition which implies either that their metabolism is simple and complex organic molecules are not required or that the organisms are able to synthesize these complex materials of the nature of vitamins, hormones, coenzymes and similar substances which must be supplied to a heterotroph. It is known that the autotrophs do synthesize required growth factors which must be supplied to the highly differentiated heterotrophs.

Many of the less highly differentiated heterotrophic bacteria synthesize a considerable number of their required factors. Werkman and Sunderlin (1928) have shown that a large number of heterotrophic microorganisms are able to synthesize thiamin. West and Wilson (1938) have shown that the root nodule organism, *Rhizobium trifolii* synthesizes thiamin and riboflavin in a purified basal medium in quantities sufficient to maintain normal growth.

Certain species, however, are unable to synthesize specific growth factors. The stimulating action of protein hydrolysates on the production of acid by the propionic acid bacteria was found by Tatum, Wood and Peterson (1936) to be due in part to thiamin. Knight (1937) showed that thiamin and nicotinic acid together replace the "staphylococcus growth factor" necessary for the growth of *Staphylococcus aureus*. Pyrimidine and thiazole could be used in place of thiamin. *Aerobacter aerogenes* and *Escherichia coli* synthesize their thiamin requirements so readily that they can not be depleted by growth in a thiamin-free medium, whereas the more fastidious *Propionibacterium pentosaceum* is readily depleted by growing in a vitamin-deficient medium (Silverman and Werkman, 1939*a*, 1939*b*, 1939*c*; Wood, Andersen and Werkman, 1938). The addition of thiamin greatly stimulates the growth. If the organism is continued in the thiamin-deficient medium for several transfers, it rapidly acquires the ability to synthesize thiamin. Silverman and

Werkman (1939*a*, 1939*b*, 1939*c*) have shown that the anaerobic dissimilation of several substrates, including glucose and lactic acid, by the propionic acid bacteria is stimulated by thiamin but that neither the thiazole nor the pyrimidine fraction alone replaces thiamin as a growth factor or metabolic stimulant. Thiamin is essential in the form of its pyrophosphate as the coenzyme of pyruvic acid dehydrogenase and also as cocarboxylase in the decarboxylation of pyruvic acid. In similar manner with respect to cozymase (coenzyme I), essential in the breakdown of glucose, bacteria possessing an autotrophic or facultative metabolism appear to synthesize their requirements (unpublished data, Scott and Werkman), whereas the pathogen, *Hemophilus parainfluenzae*, is unable to do so (Lwoff and Lwoff, 1937). For reviews the reader is referred to Koser and Saunders, 1939; Yanke, 1939; and Knight, 1936.

<div align="center">AUTOTROPHIC BACTERIA</div>

Chemosynthetic Autotrophs

An autotroph has been defined as an organism able to grow and multiply in an inorganic medium and to use CO_2 as the only source of carbon; a heterotroph, on the other hand, requires a source of carbon more complex than CO_2. Until the discovery of heterotrophic assimilation of CO_2 by Wood and Werkman in 1935, it was believed that heterotrophs were unable to fix CO_2. As knowledge of bacterial physiology increases, it becomes apparent that many of the definitions employed in the science, which have been considered fundamental and quite rigid, are losing in exactness of meaning. In the light of present knowledge, there is no necessary reason for assuming any difference in the manner of initial utilization of CO_2 by autotrophs and heterotrophs, and thus the distinction between the two groups fades. It was for this reason that a spectrum of bacterial nutrition was drawn earlier; however, retention of the terms autotroph and heterotroph is a matter of convenience.

To show further the gradual transition which occurs in passing from a typical autotroph to a heterotroph, *e.g.*, from *Thiobacillus thioxidans* to *Neisseria gonorrhoeae* the organism described by Weiringa (1940) may be referred to. It uses CO_2 and H_2 to form acetic acid but to do this it requires an extract of Dutch mud. Although the organism would appear to function as an autotroph, doubt is thrown on this status by its use of the mud extract. Since

it is not known what the constituent is in mud which is necessary for growth in an otherwise inorganic medium, it is not possible to allocate Weiringa's organism definitely and rigidly to the autotrophs. Furthermore, it is known that it can utilize glucose. Another step in the direction of heterotrophism occurs with those bacteria which assimilate CO_2 but utilize neither an inorganic reductant such as H_2S or H_2 but an organic donator of hydrogen. Here the process of CO_2 assimilation is substantially the same as before except that the hydrogen to reduce the CO_2 has been furnished by an organic compound, otherwise the latter plays no other rôle than that of a hydrogen donator and can be, in some cases, replaced by an inorganic donator.

It is likely that heterotrophism consists in the loss of ability by an organism to synthesize certain molecular structures necessary in metabolism. This does not mean, however, that certain heterotrophs may not possess the ability to carry out syntheses impossible to autotrophic forms. The fixation of nitrogen by *Azotobacter* and *Rhizobium* is a case. Both are heterotrophic in that complex carbon compounds, such as glucose, are required. It is not known why heterotrophs require complex carbon. At any rate the close relationship between the heterotroph and the autotroph is becoming increasingly clear.

It will be convenient to arrange some of the better known chemosynthetic autotrophs as in Table 1.

An interesting organism is described by Happold and Key (1937), which oxidizes ammonium thiocyanate: $NH_4CNS + 2O_2 + 2H_2O \rightarrow (NH_4)_2SO_4 + CO_2 + 220,000$ cal. It can be grown in a synthetic medium using the thiocyanate as the sole source of C and N.

Among the better known aerobic chemosynthetic autotrophs are *Thiobacillus thioparus* and *T. thiooxidans,* discovered by Waksman and Joffe (1922) in soils. The metabolism of this group was carefully studied by its discoverers and by Starkey (1925*a*, 1925*b;* Waksman and Starkey, 1922). *T. thiooxidans* will grow at a pH of 0.6, obtaining its growth energy solely from the oxidation of sulphur to sulfate. The organism is strictly autotrophic, carbon dioxide forming the sole source of carbon. Ammonium salts are the best source of nitrogen.

Among the higher forms, autotrophic bacteria oxidizing sulphur or its compounds are *Beggiatoa, Thiothrix* and *Thiocystis.*

TABLE I

CHEMOSYNTHETIC AUTOTROPHIC BACTERIA

Name	N source	C source	Energy source		Autotrophism	O_2-relationship
			Oxidation of	Reduction of		
Nitrosomonas, Nitrosococcus ...	NH_4	CO_2	NH_4	CO_2, O_2	Obligate	Aerobic
Nitrobacter ...	NO_2	CO_2	NO_2	CO_2, O_2	Obligate	Aerobic
Beggiatoa, Thiothrix, Thioploca .	NH_4	CO_2	H_2S	CO_2, O_2	Obligate (Facultative?)	
Thiobacillus thioparus ...	NH_4 NO_3	CO_2	$H_2S, S_2O_3,$ S	CO_2, O_2	Obligate	Aerobic facultative
Thiobacillus novellus ...	NH_4	CO_2	S_2O_3	CO_2, O_2	Facultative	Aerobic
Thiobacillus thiooxidans ...	NH_4	CO_2	S, S_2O_3	CO_2, O_2	Obligate	Anaerobic
Thiobacillus denitrificans ...	NH_4	CO_2	S	CO_2, NO_3	Obligate	Aerobic
Hydrogenomonas ...	NH_4	CO_2	H_2, CO	CO_2, O_2	Facultative	
Carboxydomonas oligocarbophila .	NH_4	CO_2	CO	CO_2	Facultative	Aerobic
Methanomonas methanica ...	NH_4	CO_2	CH_4	CO_2, O_2	Facultative	
Didymohelix, Sideromonas, Leptothrix, Crenothrix ...	NH_4	CO_2	$\overset{++\ \ +}{Fe, Mn}$	CO_2	Facultative, and obligate	

Perhaps the best known of the obligate aerobic chemosynthetic autotrophs are the nitrifying bacteria which oxidize ammonia (*Nitrosomonas* and *Nitrosococcus*) and nitrite (*Nitrobacter*) to nitrate. The process was shown to be biological by Schloesing and Muntz in 1877, who believed it took place in two stages. The work of Winogradsky (1890, 1891*a*, 1891*b*) led to the isolation of nitrifying bacteria on silica jelly Petri plates, and to a clear recognition that two types of organisms were involved. The two types were isolated from all nitrifying soils by repeated sub-culture in media containing ammonia but no nitrite and media containing nitrite but no ammonia. The nitrifying bacteria are extremely sensitive to unfavorable environment, particularly pH and the presence of certain organic compounds. The optimal pH for the oxidation of ammonia lies in the range of pH 8.5–8.8 (Meyerhof, 1917) and for the oxidation of nitrite between pH 8.4 and 9.3. Meek and Lipmann (1922) found that organisms isolated from peat soils at pH 4.6 nitrify at a pH as low as 4.1.

A number of reports in the literature purporting to show the existence of nitrifying bacteria capable of utilizing organic compounds have been seriously questioned, particularly by Winogradsky (1933), (however, see Boltjes, 1934).

Certain bacteria, particularly of the genus *Hydrogenmonas,* are able to oxidize molecular hydrogen and to use the energy for their needs. Such forms appear to be widely distributed in the soil, especially in such places as swamps where large quantities of hydrogen are available as the result of anaerobic processes.

Facultative heterotrophic forms exist which function as autotrophs but can use more complex compounds as sources of either nitrogen or carbon or both, and facultative autotrophs which prefer complex sources of nitrogen and carbon but are still able to use inorganic sources. Perhaps these two groups should be recognized as one, *i.e.,* the facultative forms.

It is of interest that Leeuwenhoek in 1680 observed organisms living in the absence of air (Beijerinck, 1913; Dobell, 1932). The great significance of Leeuwenhoek's discovery of the existence of anaerobes did not deter later investigators from placing undue emphasis on the rôle of oxygen in the metabolism of the cell; thus after Priestly's discovery of oxygen in 1774, Lavoissier concluded that it was essential for all life. The observations of Leeuwenhoek had been disregarded.

The anaerobe developed a series of reversible graded energy systems which released energy for use of the organism in convenient quantities, and this resulted in a smooth, even flow. With increasing differentiation the number of oxidation-reduction systems employed by a cell became greater, and successive systems involved more components to yield a smoother flow of energy. Among the first aerobes probably were those which used oxygen directly as a hydrogen acceptor. Such an aerobic oxidation mechanism would result in a sudden and uneconomic release of energy. It would be interesting to determine the extent of these simple oxytropic systems among the bacteria. If such systems do still exist, they must be disappearing with specialization and differentiation. More efficient, yet more specialized, types of respiration employ hydrogen carriers such as respiratory pigments and flavoproteins, and still more highly differentiated types employ the cytochrome system requiring two or more carriers and oxidases.

Photosynthetic Autotrophs

View of Engelmann. One of the early distinct steps in differentiation may have occurred in the origin of the photosynthetic bacteria. Certain bacteria accepting the possibilities offered by radiant energy may have used chemical energy to synthesize the first photosynthetic bio-energy transformer. In this case, sunlight is the indirect source of the energy to reduce CO_2, and during the process, H_2S is oxidized to sulfur or its oxides.

The first evidence for the existence of a photosynthetic process among bacteria was due to the work of Engelmann in 1883 from studies in the physiology of certain red pigmented (purple sulfur) bacteria. Engelmann showed that these bacteria possessed a well defined absorption spectrum, and that they congregated in portions of the spectrum identical with those absorbed. Engelmann concluded that the pigment played an essential rôle in the metabolism of the bacteria. Assuming the existence of a photosynthetic process, it would be, at first sight, logical to expect a liberation of molecular oxygen, and with this point in view Engelmann seemingly obtained positive results in 1888. Inasmuch as ordinary methods of demonstrating the liberation of oxygen in the presence of light were unsuccessful, Engelmann used certain bacteria particularly susceptible to the presence of oxygen. He found that motility in these bacteria

was greatly stimulated when they were placed with purple bacteria under a cover glass sealed with vaseline. He found similar results when green algae replaced the purple bacteria, and concluded, since it had been adequately demonstrated that the typical chlorophyll-bearing plants liberated oxygen when exposed to light, that a stimulation of the oxygen-sensitive bacteria in the presence of the purple sulfur bacteria was due to the liberation of oxygen by the latter.

Engelmann (1888_2) was convinced of the photosynthetic behavior of his organisms because of (a) their phototactic nature, (b) restriction of rapid growth to conditions of proper illumination, and (c) chemotactic behavior, involving accumulation of colorless spirilla around illuminated purple sulfur bacteria. The marked absorption by the bacteria in the infra-red region of the spectrum together with their accumulation in accordance with their absorption bands, led Engelmann to the conclusion that the liberation of oxygen by plants was not limited to the visible region of the spectrum. Although correct in his main thesis of the photosynthetic behavior of the purple bacteria, Engelmann was in error with regard to the liberation of oxygen. It is probable that the congregation of the "oxygen sensitive" bacteria around the purple bacteria in Engelmann's test was due to chemotactic influences such as reduced H_2S concentration or the positive effect of other products formed by the purple bacteria.

The phototactic accumulation of the purple bacteria in the infrared region and their absorption bands in this region convinced Engelmann that "the idea, heretofore considered strictly valid, that the evolution of oxygen by all plants is limited to the action of visual radiation, is wrong" and that the dark rays are effective in causing assimilative processes (*cf.* Van Niel, 1941).

View of Molisch. Molisch (1907), however, insisted that there was no evidence for the liberation of oxygen or even the assimilation of CO_2. Molisch's attempt to demonstrate the liberation of oxygen by purple bacteria grown in the light proved unsuccessful. He grew the organisms in a closed arm fermentation tube and in shake cultures, and even repeated Engelmann's work, to no avail. Although Molisch was of the opinion that his purple bacteria were not photosynthetic, he nevertheless realized that his experiments, failing to show the liberation of oxygen, did not constitute proof that CO_2 was not utilized. Molisch's conclusions were particularly influenced by

his observation that the non-sulfur purple bacteria with which he worked required organic carbon for their growth. A direct result of his work was the recognition of the Athiorhodaceae which do not utilize H_2S.

Among the pigmented (photosynthetic) sulfur bacteria are those with an orange-red pigment (bacteriopurpurin) and the group known as the green bacteria. The former oxidize hydrogen sulfide to sulfates, whereas the green bacteria are capable of oxidizing sulfide only so far as sulfur.

Much of the difficulty in explaining the metabolism of the sulfur bacteria lay in two principal facts: the various investigators were not working with the same organisms as a group, and the brilliant investigations of Winogradsky on the chemosynthetic autotrophs wielded an undue influence on the conclusions regarding the pigmented sulfur bacteria. Inasmuch as the purple sulfur bacteria converted H_2S into sulfate, it was concluded that their metabolism was similar to that of the non-pigmented forms, which also converted hydrogen sulfide into sulfate.

View of Winogradsky. Meanwhile, Winogradsky (1887, 1888*b*) had hit upon the explanation of the presence of sulfur granules which occur in the cells at times. Their presence was dependent upon hydrogen sulfide in the medium and its oxidation to free sulfur which in turn was oxidized to sulfate and excreted into the medium. Winogradsky believed that the sulfur served as a reserve supply of oxidizable material to provide energy. The oxidation of hydrogen sulfide and sulfur replaced that of the organic materials which characterizes most organisms. Winogradsky's concept led to the recognition of the chemoautrophic bacteria.

Winogradsky's experiments were carried out with the non-pigmented chemosynthetic bacteria, and only a few experiments were conducted with pigmented (photosynthetic) forms. The similarities between the two types, *i.e.,* oxidation of hydrogen sulfide, accumulation of sulfur granules followed by their disappearance when the hydrogen sulfide was no longer present, and the appearance of sulfate, led Winogradsky to proclaim that physiologically the two types were fundamentally alike.

Skene (1914) proved that purple bacteria can grow in an inorganic medium containing H_2S under anaerobic conditions, and resorted to Winogradsky's hypothesis that green sulfur bacteria

furnish oxygen. Bavendamm (1924) made this assumption appear highly improbable by using cultures of the purple bacteria which did not contain the green bacteria. Buder (1919) pointed out that the difficulty seemed to lie in the fact that investigators were inclined to ascribe an assimilative function to the pigment, and at the same time failed to consider Winogradsky's observations on the oxidation of the hydrogen sulfide as an essential link in the metabolism. Buder saw no objection to accepting the simultaneous occurrence of photosynthesis and chemosynthesis. He pointed out that the assumption of a photosynthetic activity is almost a necessary consequence of observed facts, differing from normal typical photosynthesis of green plants in that the oxygen liberated is used by the bacteria for the oxidation of hydrogen sulfide. Buder discussed the possibility of a purely chemosynthetic process and pointed out the superiority of the purple bacteria over the colorless forms, inasmuch as the former, under anaerobic conditions, he thought, provided themselves with necessary oxygen by a photosynthetic utilization of CO_2.

The specific difficulty here lies in explaining the need for the existence of two independent processes, namely, photosynthesis and chemosynthesis. Although there is no objection particularly to the assumption of the existence of these two independent systems side by side, and acting independently, it would mean, however, that the organisms capable of assimilating CO_2 by means of photosynthesis should be able also to develop in a medium free from H_2S, and this obviously is not true. As pointed out by van Niel (1931), it might be possible to assume that only part of the necessary CO_2 is reduced by photosynthesis, and the chemosynthetic action would have to furnish the additional supply. Van Niel calculates on the basis of Winogradsky's data for the colorless sulfur bacteria, that it would require the reduction of 80 molecules of CO_2 photosynthetically in order to liberate sufficient oxygen necessary in the process to provide the energy for the chemosynthetic reduction of 1 molecule of CO_2. It appears obvious that there is no necessity for assuming the existence of an independent chemosynthetic reaction. In fact, such an assumption would be absurd; therefore, van Niel concludes that the oxidation of H_2S as an independent source of energy is highly improbable, and another explanation must be found for the fact that there is no photosynthetic activity in the absence of hydrogen sulfide.

View of van Niel. The brilliant investigations of van Niel (1931), initiated in 1923 in Kluyver's laboratory in Delft, have led the way to a clearer understanding of the metabolism of the purple sulfur bacteria, and the reader interested in bacterial photosynthesis is referred to van Niel's (1941) excellent review.

The organisms which have been under investigation and frequently confused, may be grouped and briefly characterized for convenience in following developments in our knowledge:

Photosynthetic

1. Purple, sulfur bacteria (Thiorhodaceae, Molisch) anaerobic, develop in H_2S media readily, and oxidize inorganic sulfur compounds to sulfate with reduction of CO_2. H_2S can be replaced by certain H_2-donators, *e.g.*, lower fatty acids, dibasic acids or molecular hydrogen. Growth factors not required.
2. Purple, non-sulfur bacteria (Athiorhodaceae, Molisch). Organic substances and molecular hydrogen serve as H_2-donators; CO_2 reduced. Growth factors required. Certain species oxidize inorganic sulfur compounds.
3. Green bacteria. Occur in H_2S media. CO_2 reduced, H_2S being oxidized to free S. Growth factors not required.

Chemosynthetic 1. Colorless, sulfur bacteria. Oxidize sulfur compounds to sulfates. CO_2 reduced. Aerobic.

It was at this time that van Niel (1931) applied the ideas of Wieland (1912, 1913, 1922*a*, 1922*b*, 1925), Oppenheimer (1926) and particularly Kluyver and Donker (1926) on biological oxidation as a hydrogen transfer, to the explanation of the metabolism of the purple sulfur bacteria. Photosynthesis may be empirically represented by the equation $CO_2 + H_2O = CH_2O + O_2$. It is a typical biological oxidation-reduction. CO_2 is reduced, reduction is the addition of hydrogen, and this implies a source of hydrogen, that is, a hydrogen donator. This means that in typical photosynthetic reactions the hydrogen of the water molecules is sufficiently activated to be transferred to CO_2. Now, as van Niel pointed out, it is equally conceivable that in organisms other than typical green plants, water may not serve as the hydrogen donator but may be replaced or complemented by some other molecule, and we may represent photosynthesis, in general, according to the following equation:
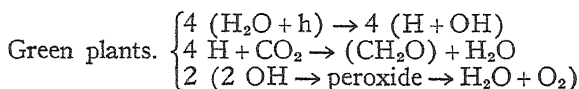
$$CO_2 + 2 H_2A = CH_2O + H_2O + 2 A$$

In 1926 Kluyver and Donker suggested that with the purple sulfur bacteria, H_2S functions as a hydrogen donator, replacing H_2O

in the typical green plant photosynthesis. Baas-Becking (1925) and Baas-Becking and Parks (1927) made a similar suggestion. Buchanan and Fulmer (1928) reaffirmed the suggestion; they say, "with the colored forms, however, it is not improbable that photosynthesis may be of importance and that the energy secured from light may be used in the decomposition of the sulphides."

Now if chemosynthesis and photosynthesis were independent processes, there would be no necessary quantitative relationship between the final products of photosynthesis. On the other hand, if H_2S is regarded as a hydrogen donator in the photosynthetic process, a very definite quantitative relationship will exist between reduced CO_2, H_2S and H_2SO_4. The experimental results of van Niel (1931) fully substantiated this latter conclusion.

Green plant photosynthesis would involve the photochemical splitting of water with the aid of chlorophyll and unknown enzymes; $HOH +$ radiant energy $\rightarrow$ "H" + "OH," the "H" going to reduce the CO_2. The final answer to the product or products formed by this reduction is not at hand. This concept may be represented by the equations (van Niel, 1941):

$$\text{Green plants.} \begin{cases} 4\ (H_2O + h) \rightarrow 4\ (H + OH) \\ 4\ H + CO_2 \rightarrow (CH_2O) + H_2O \\ 2\ (2\ OH \rightarrow \text{peroxide} \rightarrow H_2O + O_2) \end{cases}$$

There is no reason for assuming that the photochemical reaction in purple bacteria differs in principle from that in green plants, except as pointed out by van Niel (1941) who suggests that since the process with the purple bacteria occurs at longer wave lengths, the energy available to the bacteria would be less. This may make it impossible for the "OH" to rearrange into a peroxide and permit the spontaneous regeneration of oxygen and water ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$). The "OH" then could regenerate water only with the assistance of a reducing system, $e.g.,\ 2\ OH + H_2S \rightarrow S + 2\ H_2O$. The dependence of bacterial photosynthesis on the presence of suitable hydrogen donators, $e.g.,\ H_2S$ thus becomes understandable.

Bacterial photosynthesis may be represented as follows (van Niel, 1941):

$$\text{Purple bacteria.} \begin{cases} 4\ (H_2O + h) \rightarrow 4\ (H + OH) \\ 4\ H + CO_2 \rightarrow (CH_2O) + H_2O \\ 2\ (2\ OH + H_2A \rightarrow 2\ H_2O + A) \end{cases}$$
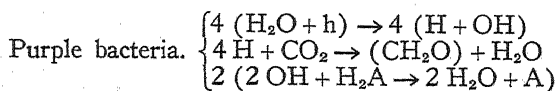
H_2A is usually H_2S although molecular hydrogen, organic donators of hydrogen, such as fatty acids, or sulfur oxides may serve depending on the species of purple bacterium used.

It is apparent that H_2S does not replace H_2O according to the concept of van Niel but rather complements it. The reduction of CO_2 is the same in both cases, *i.e.,* by the hydrogen which has been split from the water by the action of radiant energy. van Niel does not claim that the reduction of CO_2 yields carbohydrate by direct reduction but refers to the Wood and Werkman reaction in which pyruvic acid adds to CO_2 to form first oxaloacetic acid and then succinic acid, as an interesting example of what may occur, *i.e.,*

$$CO_2 + HCH_2COCOOH \rightarrow COOHCH_2COCOOH$$
$$COOHCH_2COCOOH + 4 \ H \rightarrow COOHCH_2CH_2COOH + H_2O$$
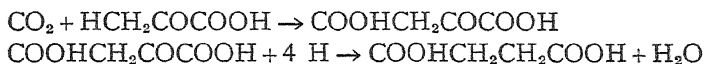
van Niel points out that if CO_2, itself, were the immediate hydrogen acceptor, one would expect formic acid to be the first reduction product, and states that unpublished work "speaks decidedly against the direct reduction of CO_2." It is seen that the function of the light is to form "active hydrogen" to reduce CO_2; from here on photosynthesis bears a close analogy to non-photosynthetic reduction of CO_2. Green plant photosynthesis comprises a series of photo-chemical and dark reactions in which the former is the decomposition of water. This manner of considering both green plant and bacterial photosynthesis and bacterial reduction of CO_2 in the dark, permits a close correlation of all three processes and a better understanding of each individually. The reduction of CO_2 in the dark will be discussed under heterotrophic assimilation.

HETEROTROPHIC BACTERIA

The heterotrophic bacteria may be defined in general terms as organisms requiring more complex (reduced) sources of carbon than CO_2. For purposes of discussion, we may arrange the heterotrophic bacteria into arbitrary groups depending upon the complexity of the nitrogen source. In one medium an organism may use ammonium salts as a source of nitrogen, whereas in another medium the same organism may fail to grow with ammonium salts as the source of nitrogen. We must visualize the specialization of the bacteria, wherein with each step in differentiation, the organism may lose its ability to synthesize certain required growth factors. In a

medium devoid of these essential growth factors the nitrogen in ammonium salts may not be available to the organism, whereas in a medium in which these factors are supplied, growth would occur. Again, still another behavior of bacteria precludes the possibility of rigidly grouping them. The culture may be unable to utilize the nitrogen in a certain medium; however, sufficient multiplication may be permitted by the use of nitrogen from dead organisms to enable the culture to grow and multiply, and to again acquire the ability to synthesize the needed factor. This type of behavior is shown by the propionic acid bacteria (Silverman and Werkman, 1939*b*). Grown in a thiamin-rich medium, *Propionibacterium pentosaceum* fails to synthesize its vitamin; however, when transferred to a medium containing just sufficient thiamin to permit slight growth, the organism acquires the ability to synthesize it.

Utilizing Elementary Nitrogen

With these considerations in mind we may first group the bacteria utilizing elementary nitrogen, such as species of *Azotobacter,* the non-symbiotic nitrogen-fixing forms, *Rhizobium,* the symbiotic root nodule bacteria, among the aerobes, and *Clostridium pastorianum* among the anaerobes.

Utilizing Ammonium Ions

The second group of heterotrophs comprises organisms which can use ammonium ions and do not require more complex forms of nitrogen. The utilization of the ammonium ion here refers to its use without the addition of vitamins or growth factors, *i.e.,* in a simple medium, such as one composed of glucose, ammonium sulfate, phosphate and the necessary additional inorganic salts or ions generally present in tap water.

Examples of heterotrophic organisms utilizing ammonium nitrogen are:

Eubacteriales: Rhizobiaceae; Pseudomonadaceae; Acetobacteriaceae; Azotobacteriaceae; Enterobacteriaceae, particularly the genera *Escherichia* and *Aerobacter;* Bacillaceae, *Bacillus.*

Actinomycetales: Actinomycetaceae, in part, *Actinomyces, Proactinomyces.*

A number of the more fastidious heterotrophs may utilize the ammonium ion in the presence of complex nitrogenous compounds,

vitamins or similar growth factors. This is true of certain species of *Clostridium* and *Propionibacterium*.

Utilizing Amino Acids

In the third group may be placed those organisms requiring amino acids as a source of nitrogen. This group is, of course, very indefinite, inasmuch as organisms may dispense with amino acids and utilize ammonia in the presence of certain unknown factors.

Utilizing Complex Nitrogen or Growth Factors

In the fourth group may be placed all organisms requiring complex nitrogen or even living tissue. This group contains the Rickettsiales, Spirochaetales, viruses, and organisms of the Eubacteriales, such as certain species of the genera *Clostridium, Staphylococcus, Neisseria, Corynebacterium, Hemophilus, Lactobacillus* and *Propionibacterium,* among others.

It is interesting to speculate on the ability of this group to synthesize their vitamin requirements. Whereas the autotrophs apparently synthesize all their vitamin requirements, and the less highly differentiated heterotrophs synthesize some of their requirements, organisms of this highly differentiated group requiring complex proteins or living tissue appear to require the addition to the medium of even more of the vitamin-like factors. It is interesting to recall that animals are notably deficient in their ability to synthesize their vitamins, but in this connection, when it is recalled that factors of the nature of vitamins (or coenzymes) function as prosthetic groups with proteins, it is clear that animal tissue probably synthesizes the needed protein portions entering into the formation of their enzymes. The addition of vitamin-like substances to the diet of certain heterotrophs does result in growth and multiplication; however, such addition has not proved effective with the most highly differentiated forms. It is tempting to suggest that such forms may also lack the ability to synthesize the protein moieties of their enzymes. It is known that these proteins are extremely specific for each enzyme.

As knowledge of the nutrition of bacteria increases, and rapid progress is being made, it will be possible to grow more and more of the factidious forms in media of known composition. Such media will be simple as compared to those used today for the "strictly" pathogenic forms, *e.g.,* those requiring living tissue. A basal

medium of ammonium salts, carbohydrates, inorganic ions and necessary vitamin-like factors will provide growth of organisms for which complex proteins, serum, yeast preparations are now deemed required.   Such a medium can not be used at present because the required factors are unknown.   Considerable progress, however, is being made in determining the vitamin needs of bacteria.   The addition of relatively complex accessory factors to the basal medium will be necessary with certain organisms which can not synthesize them, *e.g.*, coenzyme I by *Hemophilus parainfluenzae,* and trypto-phane by strains of the typhoid bacillus.   The conditions of growth are important since *Staphylococcus aureus* can synthesize uracil, required for growth, when growing aerobically but not anaerobically (Richardson, 1936).   It is emphasized that classification of the heterotrophic bacteria is arbitrary and, furthermore, not exact.

MECHANISM OF INTERMEDIARY METABOLISM

General

Early progress in understanding the phenomena of fermentation and metabolism was conditioned upon the overthrow of the theory of Spontaneous Generation.   Radot, in "The Life of Pasteur," in speaking of fermentation, remarks that all was darkness, pierced in 1836 by a momentary ray of light.   The physicist, Cagniard-Latour, studying the ferment of beer called yeast, observed that it was com-posed of cells "susceptible of reproduction" by a sort of budding, and probably acting on sugar through some effect of their vegetation. Schwann (1837, 1839), to whom belated recognition is due, was making similar observations on cell behavior.   In discussing the forces which bring about changes in environment and in the cell itself, Schwann (1837, 1839) mentions alcoholic fermentation as evidence that such changes are brought about only by a living cell.

The classical investigations of Pasteur which culminated in accep-tance of the principle that fermentation is caused by a living cell, began in 1857 with his studies on yeast and on the lactic fermenta-tion.   Pasteur's views met vigorous opposition, especially from the great chemist Liebig who held firmly to the belief that fermentation was caused by a "disquietude" of the protein molecules induced by the oxygen of the air.   Pasteur showed that the presence of fermen-tation resulted in growth and multiplication of yeast cells, and further, that growth and multiplication could be brought about in

a medium containing only inorganic constituents, *i.e.,* no protein molecules to transmit the "state of disquietude" to the sugar molecules.   In the light of our present knowledge we can look back on the famous controversy of Liebig and Pasteur in calm reflection on the merits of the two views.   Pasteur was correct in that fermentation, as it occurs naturally, is due to living organisms, but that it is an act correlative with life has not been substantiated, and in this respect the views of Pasteur have had to be modified as the result of the discovery of Buchner in 1897 that fermentation took place in a cell-free press juice of yeast.   Buchner's discovery provided the techniques to explore the intermediary metabolism of microorganisms.

The metabolism of bacteria may be schematized as in Figure 3. Dissimilation may be defined in general terms as the transformation
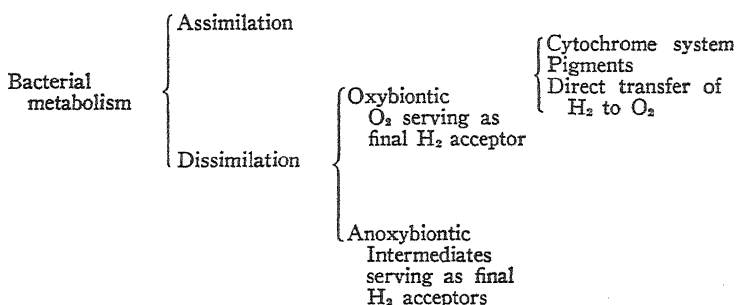
Fig. 3.   Outline of Bacterial Metabolism

of the substrate to yield energy for the use of the organism as distinguished from the endothermic changes which characterize assimilation, as in the formation of cell substance.   Dissimilation may, of course, provide certain intermediary products necessary as building blocks in assimilation.

Dissimilation may be conveniently discussed as oxybiontic and anoxybiontic.   Oxybiontic dissimilation may be defined as the transformations undergone by the substrate in which oxygen plays the rôle of final hydrogen acceptor and water is formed, whereas in anoxybiontic dissimilation oxygen does not function as a hydrogen acceptor.   In this case, intermediate products can perform the duty of acceptors; *e.g.,* ethyl alcohol may be formed from acetaldehyde, butyl alcohol from butyric acid, 2, 3-butylene glycol from acetylmethylcarbinol, or lactic acid from pyruvic acid.   The terms oxy-

biontic and anoxybiontic are used here to replace the more fre-
quently used terms, aerobic and anaerobic. Generally an organism
growing aerobically uses oxygen, and an organism growing in the
absence of molecular oxygen does not; however, this may not always
be true. It is conceivable that an organism may grow in the pres-
ence of gaseous oxygen and still not make a normal physiological
use of it as a hydrogen acceptor; the anaerobes do not have an
aerobic mechanism for the utilization of the oxygen; some may use
it unphysiologically, such as certain of the lactic acid bacteria, to
form toxic peroxide. Again, there is some evidence (Frei, 1934)
that organisms may obtain their oxygen from constituents of the
medium, *e.g.,* from nitrates; evidence at present indicates that such
organisms are using an essentially oxybiontic metabolism. It is to
be noted that the cell must have the necessary enzyme to make the
oxygen of the nitrate available, *i.e.,* to reduce the nitrate. The
metabolism of organisms using bound oxygen appears to be inhibited
by cyanide.

Frequently respiration (oxybiontic dissimilation) is classified as
"principal" or "secondary." The differentiation is not essential and
there are no techniques by which the relative proportion which each
constitutes of the whole can be determined. That susceptible to
cyanide roughly constitutes "principal respiration," and respiration
not inhibited by cyanide is classed as "secondary"; however, part of
the "secondary" may be cyanide-inhibited. A further complication
arises from the possible realignment of the relative importance of
various systems on poisoning with cyanide. A system normally of
secondary importance may assume greater importance in the pres-
ence of cyanide.

In the field of intermediary bacterial metabolism, especially in
attempting a clear elucidation of dissimilation and assimilation, we
recall the words of the physiologist Hill: "Although it is dangerous
to speculate too far, it is foolish not to speculate at all." Our
remarks will be directed specifically toward the bacterial cell, al-
though the general problem of cell physiology has received more
extensive treatment with yeast and animal cells, especially those of
muscle. There are certain technical advantages in working with
metabolic phenomena in highly specialized cells, as those of muscle,
in contrast to the bacterial cell. In muscle we are dealing with cells
which have become highly specialized and differentiated. Differ-

entiation has led to simplication of the individual cell processes, whereas the bacterial cell constitutes the individual and each cell must carry on all the functions necessary to life of an organism. Physiologically bacteria are not simple cells. In addition, bacteria must carry on all their processes under widely varying environmental conditions of pH, oxidation-reduction potential, temperature, nutrition and other factors. The bacterial cell is the street urchin of the cell world.

Inasmuch as bacterial metabolism, both dissimilation and assimilation, is dependent on oxidative processes, we shall briefly review present day concepts of biological oxidation.

Biological Oxidation

Biological oxidation manifests itself in a transfer of hydrogen (or electrons) from a donator to an acceptor. Clark (1923) defined biological oxidation as "the withdrawal of electrons from a substance with or without the addition of oxygen or elements analogous to oxygen; or as the withdrawal of electrons with or without the withdrawal of hydrogen or elements analogous to hydrogen." It is generally accepted that the rôle of oxygen is that of a hydrogen acceptor to form H_2O_2 and H_2O; that is, oxygen, as such, does not enter the molecule of the substrate. In anoxybiosis oxygen is replaced by some other suitable hydrogen acceptor. Wieland (1912, 1913, 1922a, 1922b, 1925) and Thunberg (1916, 1918, 1920) have provided us with the basic concepts of our knowledge of biological oxidation.

Enzymes of Oxidation. Inasmuch as the transfer of hydrogen is activated by specific enzymes known as dehydrogenases, we shall summarize present knowledge of the nature and behavior of the oxidation enzymes which bring about the transfer of hydrogen. So far as the enzymes important in oxidation have been examined, they have been found to consist of a protein portion and a group of lower molecular weight known as a prosthetic group, such as Cu^{++}, Cu^+, iron porphyrin, diphosphothiamin, flavinadenine dinucleotide, pyridine nucleotide and others. Both portions are specific, although members of a type (Table 2) have the same or similar prosthetic groups but differ in the protein moiety. The transfer of the hydrogen atom is reflected in the behavior of the prosthetic group, *i.e.,* it accepts the hydrogen and then passes it along to the acceptor.

TABLE 2

TYPICAL COENZYMES

Name	Type	Reaction catalyzed	Source
Cocarboxylase	Diphosphothiamin protein	Decarboxylation of pyruvic acid	Yeast, bacteria
Pyruvic codehydrogenase	Diphosphothiamin protein	Dehydrogenation of pyruvic acid	*L. delbrückii* *Neisseria gonorrhoeae*
Diaphorase	Flavoprotein Flavinphosphate	Oxidation of reduced coenzymes I and II	Yeast
Coenzyme II dehydrogenase	Flavinadenine dinucleotide	Oxidation of reduced coenzyme II	Yeast
Polyphenoloxidase	Metal protein ++ Cu	Oxidation of O-diphenols by O_2	Potato *Agaricus campestris*
Monophenoloxidase	++ Cu	Oxidation of monophenol by O_2	*Lactarius piperatus*
Anhydrase	Zinc	Liberation of CO_2 from H_2CO_3	
Catalase	Iron Fe-protoporphyrin + bile pigment hemochromogen	Liberation of molecular oxygen from H_2O_2	Yeast, bacteria
Hemoglobin	Fe-protoporphyrin	Combines with and dissociates from O_2	
Cytochrome c	Fe-protoporphyrin	Transfer of Hydrogen to O_2 in presence of its oxidase	Yeast, aerobic bacteria
Coenzyme I (cozymase)	Pyridine protein Diphosphopyridine nucleotide	Transfer of hydrogen	Bacteria, yeasts, widespread in Nature
Coenzyme II	Triphosphopyridine nucleotide	Transfer of hydrogen	Muscle, bacteria

Theorell (1935) split the riboflavin-protein enzyme complex into protein and prosthetic riboflavin groups by dialysis. The protein could be combined with flavin phosphate prepared synthetically to form an active enzyme. Green *et al.* (1940) have isolated car-

boxylase, the enzyme splitting CO_2 from pyruvic acid, from top brewers' yeast in highly purified and stable form. It is a diphospho-thiamin magnesium protein which can be resolved into its component parts by precipitating three times from ammoniacal ammonium sulfate solution. The enzyme was reconstituted from the three components. Magnesium was replaced by any divalent cation tested, *e.g.,* Mn, Fe, Ca, Cd, Zn or Co, although with varying degrees of activity. Mono- or tervalent cations were ineffective.

There is at present no universally accepted system of nomenclature to designate specific enzymes or the constituent parts of an enzyme. It has been suggested that the combination of protein and prosthetic group be called holoenzyme; the prosthetic group becomes the coenzyme and the protein the apoenzyme. The coenzyme was formerly considered to be a separate, dialyzable, thermostable substance necessary in addition to the enzyme and substrate to initiate the reaction. A coenzyme is usually an organic compound, although the term was first used by Bertrand (1897) to characterize inorganic ions (Ca and Mn) which activated plant enzymes, and is today frequently applied to inorganic ions serving as prosthetic groups, as in the case of Cu^{++} in the polyphenol oxidases. Recent use of the term dates from the pioneering work of Harden and Young (1905, 1906) who found the thermostable, dialyzable fraction of yeast juice necessary to initiate fermentation by the dialyzed residue. Confusion existed regarding the nature of yeast coenzyme (cozymase). Any dialyzable, thermostable substance stimulating the activity of yeast press-juice was considered a coenzyme, *e.g.,* ions of K, Mg, PO_4, or a hydrogen acceptor (necessary to initiate a reaction which continued by virtue of acceptors formed subsequently), anti-protease, *etc.* Remarkable progress has been made in recent years in elucidating the action of coenzymes. Our point of view regarding their nature and behavior is changing somewhat. The relationship appears to involve a union of the coenzyme and apoenzyme (protein fraction) whose firmness varies with the enzyme. In certain cases (yeast zymase) dissociation of the coenzyme from the protein readily occurs, and separation may be effected by dialysis. In other cases dissociation apparently does not occur readily with our present methods and we actually speak of such an enzyme as not requiring a coenzyme. In Table 2 are listed representative oxidation coenzymes. A coenzyme may function in one or more of several ways, *i.e.,* as a H_2-carrier, O_2-carrier, PO_4-carrier.

Coenzymes of oxidation may be grouped into types: (*a*) diphosphothiamin, (*b*) flavinnucleotide, (*c*) pyridine nucleotide and (*d*) metal. Certain coenzymes transport PO_4. Adenylic acid accepts PO_4 to become adenosine triphosphate and thus transports one or two PO_4 groups. It appears that a coenzyme constitutes a one-member bucket brigade, acting as the means to transport an atom, group or molecule from the donator to an acceptor. Although the actual change occurs on the coenzyme, the protein moiety is essential. A system comprised of donator, coenzyme and acceptor will not react until the specific protein is added. Apparently the protein alters (labilizes) or instigates the alteration of the donator in such a manner that two atoms of hydrogen (in the case of oxidation enzymes) are transferred. It is probable that the protein through one of its groups forms a union with the donator; the electrostatic forces of the donator-protein complex must be such as to loosen the hydrogen. The mechanism of this labilization is unknown at present. It is known, however, that the protein portion is highly specific, but as to the chemical structure that leads to this high specificity our knowledge is fragmentary. The reader is referred to Green (1941) for an interesting discussion of enzymes and trace substances.

The efficiency of an enzyme may be determined by the number of cycles of oxidation and reduction its molecule undergoes in a minute, a value known as the "turnover number." Catalase has a turnover number of about two and one-half million (0° C.) ; *d*-amino acid oxidase about 2,000 (38° C.) and cytochrome c about 1,500.

The nature of the cyclical change undergone by the coenzyme varies even among the oxidation enzymes. In cytochrome c a valency change $Fe^{++} \rightleftharpoons Fe^{+++} + e$, and similarly in catalase (Keilin and Hartree, 1936) the Fe atom undergoes oxidation by O_2 and reduction by H_2O_2, whereas in hemoglobin and peroxidase (Keilin and Mann, 1937) the Fe atom does not change in valency, remaining reduced in the former and oxidized in the latter. Cu^+ is the prosthetic group of the respiratory pigment hemocyanin. It remains in the cuprous condition. Hemocyanin undergoes a cyclic combination with and dissociation from molecular oxygen. In the phenoloxidases copper is also the coenzyme but here it undergoes a cyclic valency change, $Cu^+ \rightleftharpoons Cu^{++} + e$. In *Agaricus campestris* the prosthetic copper atom has a turnover number of about 70,000 (20° C.) (Keilin and Mann, 1938).

Bortels (1930) found that small amounts of molybdenum are required by *Azotobacter,* the non-symbiotic nitrogen-fixing bacteria. This discovery suggests that molybdenum forms the prosthetic group of a nitrogen-fixing enzyme.

Yeast carboxylase, which liberates CO_2 from pyruvic acid, ($CH_3COCOOH$) has a prosthetic group which is a diphospho-thiamin-magnesium compound (Green *et al.,* 1940). In this case Green has suggested that magnesium functions as a cementing material to bind the protein and prosthetic groups together and not as a catalyst, since it may be replaced by a divalent metal such as nickel, cobalt, calcium or manganese. The cyclic change undergone by the prosthetic group is unknown at present.

Zinc has been shown to be the prosthetic group of carbonic anhydrase, the enzyme which catalyzes the liberation of carbon dioxide from carbonic acid in the blood (Keilin and Mann, 1937).

The identity of several of the vitamins and certain prosthetic groups of enzymes in recent years has proved of interest. The identity of thiamin as the diphospho compound with cocarboxylase (Lohmann and Schuster, 1937; Williams and Cline, 1936), black tongue vitamin with nicotinamide (Elvehjem, *et al.,* 1937), and vitamin B_2 with flavin (Kuhn, *et al.,* 1933*a,* 1933*b,* 1934) are examples.

Frequently the prosthetic group as a whole is not needed; thus thiamin-depleted propionic bacteria require only thiamin and PO_4 to synthesize carboxylase; the protein moiety is synthesized and thiamin is united to the PO_4 to form diphosphothiamin which is combined with protein to form the enzyme. On the other hand, untrained bacteria can not synthesize either the thiazole or pyrimidine fraction to form thiamin when the other is added to the medium (Silverman and Werkman, 1939*a,* 1939*b*). They can, however, be trained to synthesize thiamin by carefully culturing in thiamin-deficient media.

Biotin has been reported as an essential growth factor for *Rhizobium radicicola* (Nilsson, Bjälfve and Burström, 1939), *Clostridium butylicum* (Snell and Williams, 1939), *Cl. sporogenes* (Peterson, McDaniel and McCoy, 1940), *Staphylococcus aureus* (Porter and Pelczar, 1940), and a strain of group A hemolytic streptococcus (Hottle, Lampen and Pappenheimer, 1941). Its mode of action is not known.

Carbohydrate Dissimilation

Before we discuss the Embden-Meyerhof-Parnas scheme of carbohydrate dissimilation, it is desirable that the reasons and evidence be advanced for its application to bacteria. From the proposal of the scheme in its original form in 1933 until 1936, it had been shown to apply only to highly differentiated animal tissue and to yeast, notwithstanding efforts to apply the scheme to bacteria. In 1936 the isolation of phosphoglyceric acid from the dissimilation of glucose by *Citrobacter freundii*, an organism of the coliform group, was reported by Werkman, Zoellner, Reynolds and Gilman (1936). Additional isolations were reported with a large series of representative genera of bacteria, *e.g., Bacillus, Propionibacterium, Aerobacter, Escherichia, Lactobacillus, Serratia, Azotobacter, Streptococcus* and *Staphylococcus* (Stone and Werkman, 1936*a,* 1936*b,* 1937; Werkman, 1936). Later Werkman, Stone and Wood (1937) suggested that although they believed the Embden-Meyerhof-Parnas scheme was chiefly responsible for carbohydrate dissimilation, possibly additional paths of breakdown were available to bacteria, since certain propionic bacteria grew well and multiplied in the presence of sodium fluoride which blocks the Embden-Meyerhof-Parnas scheme.

Additional evidence for the Embden-Meyerhof-Parnas scheme in bacterial metabolism has been presented by Utter and Werkman (1941) in their proof of the existence of the aldolase equilibrium as well as the isomerase equilibrium. Additional evidence will be presented when the scheme is discussed, although the two points given constitute strong support of the Embden-Meyerhof-Parnas scheme.

Embden-Meyerhof-Parnas Scheme. The early scheme of Embden will not be discussed. It has been markedly expanded, but in its original form contained the essential framework of the scheme in its present form as applied to muscle metabolism (Embden, Deuticke and Kraft, 1933).

The Embden-Meyerhof-Parnas scheme in its present form is given in Figure 4. The reactions consist of phosphorylations, oxidations and hydrolyses. Many are equilibrium reactions. The scheme as outlined starts with the phosphorylation of glucose, first to a monophosphate and then to a hexosediphosphate. Phosphate is probably essential in the metabolism of all cells, and accumulating

2 Glucose + adenosinetriphosphate $\xrightarrow{\text{hexokinase}}$ 2 hexosemonophosphate + adenylic acid

2 Hexosemonophosphate + adenosinetriphosphate → 2 hexosediphosphate + adenylic acid

Hexosediphosphate $\underset{\text{aldolase}}{\rightleftharpoons}$ glyceraldehydephosphate $\underset{\text{isomerase}}{\rightleftharpoons}$ dihydroxyacetonephosphate

+2 H   +PO$_4$

α-glycerophosphate   1,3-diphosphoglyceraldehyde

glycerol + H$_3$PO$_4$ −2 H

1,3-diphosphoglyceric acid + 2 adenosinediphosphate → adenosine triphosphate + 3-phosphoglyceric acid, Warburg and Christian (1939) modification

3-Phosphoglyceric acid $\underset{\text{phosphoglycero mutase}}{\rightleftharpoons}$ 2-phosphoglyceric acid

2-Phosphoglycerate $\underset{\text{enolase}}{\rightleftharpoons}$ phosphopyruvate + H$_2$O

2 Phosphopyruvate + adenylic acid → pyruvic acid + adenosinetriphosphate

Adenosinetriphosphate + 2 creatine ⇌ adenylic acid + 2 creatine phosphate

Adenosinetriphosphate → adenylic acid + 2 H$_3$PO$_4$

Diphosphoglyceraldehyde + CH$_3$CHO $\xrightarrow{\text{mutase}}$ C$_2$H$_5$OH + diphosphoglycerate

Diphosphoglyceraldehyde + pyruvate → lactate + diphosphoglycerate

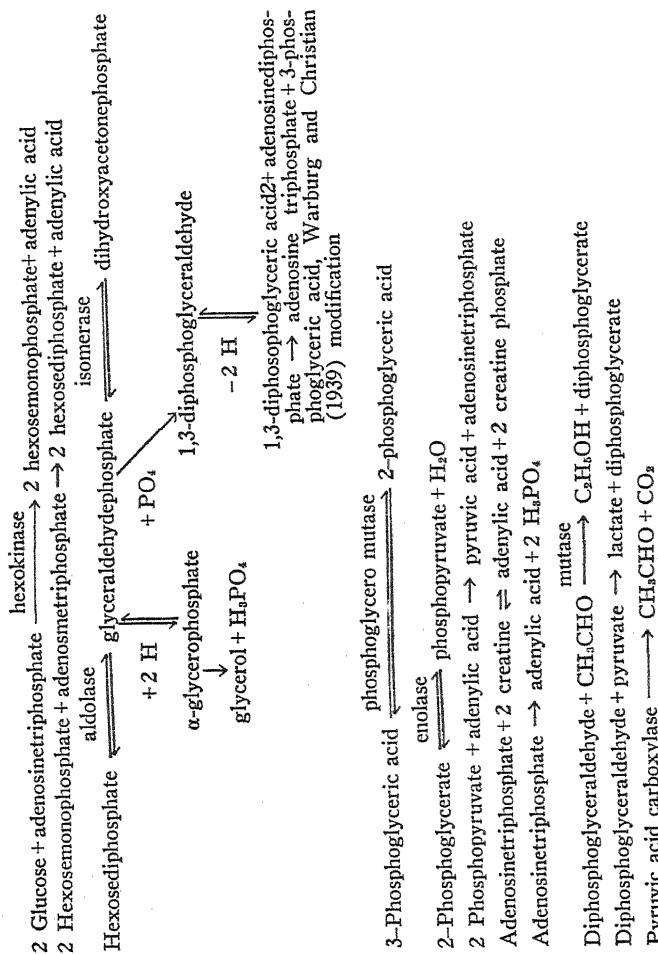Pyruvic acid carboxylase → CH$_3$CHO + CO$_2$

Fig. 4. Embden-Meyerhof-Parnas scheme of dissimilation

evidence is extending rather than restricting its rôle in forming energy-rich bonds (*cf.* Lipmann, 1941).

After the discovery of phosphorylation by Harden and Young in alcoholic fermentation of glucose, it was thought of as a process which prepared the glucose for dissimilation, *e.g.,* the introduction of the two phosphate groups to form hexosediphosphate was thought to develop lines of cleavage of the molecule into two triose phosphates. It is now clear that phosphorylation results in the accumulation of large quantities of energy at the PO_4 linkage, which becomes available to the organism. These energy-rich phosphate bonds may be referred to as energy carriers (Negelein and Brömel, 1939; Lipmann, 1940, 1941).

Whereas it was formerly believed that phosphorylation was limited to the early stages of the scheme, it has been extended to the latter stages beyond pyruvic acid. The work of Lipmann (1941) and additional evidence of Silverman and Werkman (1941) indicate that phosphorus plays an essential rôle in the dissimilation of pyruvic acid. Lipmann (1937, 1939*a,* 1939*b*) has shown that phosphate must participate in the dehydrogenation of pyruvic acid by lactic acid bacteria and the energy derived can be used to synthesize adenosinetriphosphate from free phosphate and adenylic acid; Mg^{++}, Mn^{++} or Co^{++} is required. Silverman and Werkman (1941) have shown that phosphate is required in the conversion of pyruvic acid into acetylmethylcarbinol and carbon dioxide by a cell-free enzyme preparation obtained from *Aerobacter aerogenes.*

We are much in the dark as to the phosphorylation changes taking place during the initial attack of bacteria on carbohydrates. The evidence is clear, however, that phosphorylation does occur (Virtanen, 1924, 1925; Virtanen and Karström, 1931). The uptake of inorganic phosphate by bacteria has been shown by Stone and Werkman (1936*b*) and Wiggert and Werkman (1938). Owing to our lack of knowledge as to the exact changes occurring during the initial attack on glucose, we assume it to be a phosphorylation of the glucose to hexose-6-phosphate.

There is as yet no evidence for the formation of the Cori ester (hexose-1-phosphate) (Cori and Cori, 1936, 1937) by bacteria. The ester is formed from polysaccharides by muscle and is rapidly converted into the 6-ester. The 6-monophosphate is converted into the Harden-Young ester (fructose-1-,6-diphosphate). Glycogen or

similar compounds may form first to start the dissimilation. There is evidence that glycogen is formed before dissimilation occurs by animal tissues. For cells such as muscle and erythrocytes this fact has been recognized. Willstätter and Rodewald (1937) report that yeast and leucocytes synthesize glycogen from glucose prior to the initial stage of dissimilation. Both Parnas and Willstätter have demonstrated with yeast and muscle that the attack on glycogen yields active sugar phosphates and not phosphate-free hexose (*cf.* Ostern *et al.*, 1937). Thus the first attack on glycogen is not a hydrolysis but a phosphorolysis (Parnas) in which glucosidic linkages are broken by the attachment of phosphate groups.

Assuming free hexoses to be attacked directly, the process must start with an intramolecular rearrangement of a stable hexose into a reactive form. The latter is now prepared for attack by phosphorylation. Although there are several phosphate esters formed during dissimilation, it appears that the furanoid fructose-1,6-diphosphoric acid ester is the important intermediate indicating a change from the pyranoid into the furanoid structure (*cf.* Oppenheimer and Stern, 1939).

In the Embden-Meyerhof-Parnas scheme of fermentation the furanoid hexosediphosphate occupies the important starting position, arising by phosphorylation of the hexosemonophosphate by phosphopyruvic acid. The first split occurring in the dissimilation is the formation of the equilibrium: hexosediphosphate $\rightleftharpoons 2$ triosephosphate, effected by aldolase. The two triosephosphates comprise a molecule each of glyceraldehyde phosphate and dihydroxyacetonephosphate which in turn are brought into equilibrium (shifted far to the dihydroxyacetonephosphate) by isomerase. These two systems now have been demonstrated to occur in bacterial metabolism. Using hexosediphosphoric acid and *Escherichia coli,* Utter and Werkman (1941) have shown the occurrence of the aldolase and isomerase equilibria in which about 95% of the product is dihydroxyacetonephosphate. Both dihydroxyacetonephosphate and glyceraldehydephosphate have been tentatively identified. A true equilibrium was shown to exist by demonstrating temperature and concentration effects. Warburg and Christian (1939) recently showed that glyceraldehydephosphate unites with phosphate to form 1, 3-diphosphoglyceraldehyde which after oxidation to the acid loses one phosphate group to adenosinediphosphate to become 3-phosphoglyceric acid.

According to the original view of Embden and Meyerhof, the equilibrium of triosephosphoric acid gave rise to the formation of α-glycerophosphate and 3-phosphoglyceric acid. The formation of glycerophosphoric acid has been more or less relegated to the background by more recent investigations, although there is definite evidence that glycerophosphate is formed under suitable conditions. It will be noted that the formation of glycerophosphate and 3-phosphoglyceric acid is the result of the triosephosphate undergoing a dismutation, *i.e.,* the oxidation of one molecule of triosephosphate with the simultaneous reduction of a second molecule. It was originally argued that such a dismutation occurred in the absence of a suitable hydrogen acceptor. As soon as pyruvic acid in the case of muscle or acetaldehyde in the case of yeast was formed, the reduction of triosephosphate to α-glycerophosphate no longer occurred, and in its place the pyruvic acid or acetaldehyde was reduced to lactic acid or ethyl alcohol respectively. The work of Warburg and Christian (1939) has now shown that glyceraldehydephosphate takes up an additional inorganic phosphate group to become 1,3-diphosphoglyceraldehyde. The latter is then dehydrogenated (by pyruvic acid or acetaldehyde) to 1,3-diphosphoglyceric acid which in turn donates one phosphate group to adenosinediphosphate (*cf.* Fig. 4).

The 3-phosphoglyceric acid is the characteristic intermediate of the Embden-Meyerhof-Parnas scheme. Its isolation from a fermentation or cellular dissimilation is indicative of the occurrence of the Embden-Meyerhof-Parnas scheme in the metabolism of the cell involved. The 3-phosphoglyceric acid sets up an equilibrium with 2-phosphoglyceric acid which is then transformed into phosphopyruvic acid under the influence of enolase with the loss of 1 molecule of water. The kinetics of cell metabolism after phosphopyruvic acid is formed, are not clear and require additional study. According to the accepted scheme, phosphopyruvic acid donates its phosphate group to hexosemonophosphate, the phosphate group is transported to the hexosemonophosphate by adenylic acid (adenosinemonophosphate). The adenylic acid becomes the triphosphate. The adenosinetriphosphate transforms the hexosemonophosphate into hexosediphosphate and again becomes adenylic acid. In animal tissues the triphosphate may donate phosphate to creatine with the aid of phosphorylase to form adenylic acid and creatinephosphate

which serves as a reservoir of phosphate. Evidence at present indicates that yeast does not use this method of storing phosphate, and there is no evidence of its presence in bacterial metabolism.

The widespread occurrence of adenosine phosphates in nature strongly supports phosphate transfer as a universally occurring metabolic function. The review of Lipmann (1941) should be consulted for details of phosphorylation.

Embden, Deuticke and Kraft (1933) showed that phosphoglyceric acid is fermented to pyruvic acid and phosphoric acid by muscle tissue; Antoniani (1933), and Werkman, Zoellner, Gilman and Reynolds (1936) for *Escherichia coli*:

$$CH_2OH_2PO_3 \cdot CHOH \cdot COOH \rightarrow CH_3COCOOH + H_3PO_4$$
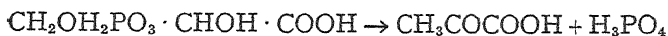
Subsequently Lohmann, Meyerhof and Kiessling showed that Embden's reaction occurred in three steps: $CH_2OH_2PO_3 \cdot CHOH \cdot COOH \rightleftharpoons CH_2OH \cdot CHOH_2PO_3 \cdot COOH \rightleftharpoons CH_2 : COH_2PO_3 \cdot COOH \rightarrow CH_3COCOOH + H_3PO_4$. The 3-phosphoglyceric acid was found to be in equilibrium with 2-phosphoglyceric acid which in turn was in equilibrium with phosphopyruvic acid which split into pyruvic acid and phosphoric acid.

Parnas, Ostern and Mann (1934) observed that the transfer of phosphate from phosphoglyceric acid ultimately to adenylic acid occurs in muscle to form the adenosinetriphosphate containing energy-rich phosphate bonds. The transfer occurs only in the presence of adenylic acid to accept the phosphate (Lohmann and Meyerhof, 1934) *via* phosphopyruvic acid. Meyerhof and Kiessling (1935) later isolated the 2-phosphoglyceric acid. In the absence of adenylic acid an equilibrium mixture of all three products occurs. Lohmann and Meyerhof were now able to show that sodium fluoride, known to be a powerful inhibitor of fermentation, exerted its effect on enolase, thus preventing the breakdown of 2-phosphoglyceric acid into phosphopyruvic acid.

Only recently have reactions been found which explain the initial introduction of inorganic phosphate into organic combination. Cori (1939) and Parnas (1937) have established phosphorolysis of glycogen, and Negelein and Brömel (1939) and Lipmann (1940) have shown phosphate addition to carbonyl double bonds. The reader is referred to the excellent reviews by Cori (1939) and Lipmann (1941). It is assumed by Cori that phosphorylation of glu-

cose occurs prior to the synthesis of glycogen. The entry of phosphate into carbonyl groups is made probable by the work of Negelein and Brömel (1939), Lipmann (1940) and Silverman and Werkman (1941) because of the necessity for the presence of phosphate in certain enzymatic dehydrogenations of carbonyl compounds and in the formation of acetylmethylcarbinol from pyruvic acid. The nature of the intermediate organic phosphate is not known at present, although Lipmann suggests that it is acetylphosphate.

Pyruvic acid is a cardinal intermediate in carbohydrate dissimilation. It occurs in the carbohydrate metabolism of many, if not all types of cells, both animal and plant.

Pyruvic acid may yield many of the products of the bacterial dissimilation of carbohydrate, such as formic, acetic, propionic, butyric, succinic, lactic and fumaric acids, ethyl, butyl and isopropyl alcohols, glycerol, acetylmethylcarbinol, 2,3-butylene glycol, acetone, carbon dioxide, and hydrogen. Pyruvic acid may also serve in the formation of amino acids. In muscle glycolysis the pyruvic acid is reduced to form lactic acid; with yeast it is first decarboxylated to acetaldehyde and CO_2; the former is normally then reduced to ethyl alcohol. If the yeast fermentation is carried out at relatively alkaline pH levels, a dismutation of the acetaldehyde occurs to form equimolar quantities of ethyl alcohol and acetic acid (Neuberg type III fermentation). If sulfite is added to a normal yeast fermentation of glucose, it forms an addition-compound with the intermediately occurring acetaldehyde, thereby preventing the reduction of the latter to ethyl alcohol. Hydrogen is then forced to reduce glyceraldehyde to form glycerol (Neuberg, II fermentation). In lactic acid bacteria, Lipmann (1939*b*, 1939*c*) has shown that phosphate must participate in the dehydrogenation of pyruvic acid and the energy derived can be used to synthesize adenylic acid pyrophosphate from free phosphate and adenylic acid. With bacteria the reactions involving pyruvic acid are complex. Lactic acid (*d*, *l*-, or *dl*) may be formed by many groups, *i.e.*, *Lactobacillus, Streptococcus* and *Bacillus*. *Sarcina ventriculi* (Smit, 1930) possesses an essentially alcoholic mechanism, and a mixed lactic acid-alcoholic type of dissimilation is shown by *Thermobacterium mobile* which converts glucose into lactic acid (about 7%), ethyl alcohol and CO_2 (45% each) (Hoppenbrouwers, 1931).

The mechanism of the anoxybiontic dissimilation of pyruvic acid

by bacteria is uncertain. It may undergo dismutation to form lactic acid and acetic acid + CO_2. This reaction probably occurs with *Lactobacillus* (heterofermentative forms), *Staphylococcus* and *Neisseria*. Other intermediate products may function as hydrogen acceptors in place of pyruvic acid to form their reduction products in place of lactic acid. Other reactions have been suggested to account for the formation of certain products such as formic acid, acetylmethylcarbinol, 2,3-butyleneglycol, however, it appears that these reactions will require proof before they can be accepted. In this category is the hydroclastic reaction ($CH_3COCOOH + H_2O \rightarrow CH_3COOH + HCOOH$) and that responsible for the formation of acetylmethylcarbinol by condensation of two molecules of acetaldehyde.

Our knowledge regarding the oxybiontic dissimilation of pyruvic acid is in the developmental stage. The citric acid cycle of Krebs has provided us with one explanation.

The principle is generally accepted that anoxybiosis is the important preparatory step for terminal oxybiontic dissimilation, although the possibility of a different type of carbohydrate dissimilation has been suggested by attempts to demonstrate a respiration which is independent of anoxybiontic dissimilation by Lundsgaard (1930, 1932), according to whom iodoacetic acid suppresses the anoxybiontic metabolism of yeast without appreciably affecting the oxybiontic reaction. There is also the work of Trautwein and Wassermann (1931), and Trautwein and Weigand (1931). On the other hand, Hoogerheide (1935) has shown that yeast is able to oxidize only those carbohydrates which are fermented. The problem of a carbohydrate dissimilation not involving the usual products of fermentation has not been clarified. Such a metabolism is possible in the case of plants, particularly certain fungi (Boysen-Jensen, 1931).

It is, however, quite certain that pyruvic acid is usually the essential substance of the beginning of oxybiontic dissimilation in normal cells.

Protein Metabolism

Microorganisms may obtain their nitrogen from various sources, inorganic or organic. The autotrophic bacteria obtain their nitrogen requirements for multiplication and non-proliferative metabolism from salts of ammonia, nitrates, nitrites, and other nitrogen-

containing salts.   Many heterotrophic bacteria require complex sources of nitrogen.   It is known that certain bacteria which are generally considered to require a complex source of nitrogen, such as a protein, can, however, use ammonia as their source of nitrogen if accessory factors are added to the medium.   Thus *Bacillus dextrolacticus,* described by Andersen and Werkman (1940), is able to grow and multiply in a medium containing ammonium sulfate as the source of nitrogen if an ether extract of yeast extract is added in traces.   Organisms able to grow on simple amino acids probably use the nitrogen obtained by deamination.   Some organisms which grow relatively slowly on ammonia media or even amino acid-containing media are apparently able to synthesize their amino acids or polypeptides only slowly, and supplying the organisms with a more complex nitrogen source will result in more rapid multiplication.   Bacteria have proved themselves to be quite adaptable to the synthesis of components necessary in their metabolism from simple compounds.   The work of Fildes, Gladstone and Knight (1933) has shown that certain exacting strains of the typhoid bacillus grow in ammonium chloride medium with glucose, citrate and salts, provided tryptophane is added.   The concentration of tryptophane required for optimal growth (equivalent to 0.00005% nitrogen) indicates clearly that the trytophane does not supply nitrogen for protein synthesis but probably does furnish a group or structure that the organism is unable to synthesize.   By growing the exacting strains on simple media with decreasing concentrations of tryptophane, it was found possible to modify the strain so that it eventually grew on a purely synthetic medium with nitrogen supplied by ammonium chloride and carbon by glucose. Likewise, in the case of *Staphylococcus aureus,* Gladstone (1937) found that tryptophane was essential in four of 25 strains studied. Wood, Geiger, and Werkman (1940), working with heterofermentative lactic acid bacteria, found that tryptophane was essential for one culture, *Lactobacillus Buchneri,* but that *Lactobacillus mannitopoeus* and *Lactobacillus lycopersici* could dispense with tryptophane in the medium.   Riboflavin, thiamin and factors present in the ether extract of yeast extract were necessary for maximal growth of these bacteria in an amino acid medium.   No attempt was made to adapt these bacteria to an ammonium salt medium.   The exact mechanisms by which ammonium salts are synthesized into protein are unknown.

Dissimilation of Amino Acids. Bacteria may utilize proteins to provide building blocks for the synthesis of body proteins and other nitrogen-containing constituents present in the cell, such as coenzymes, and to provide energy when necessary.

It is generally agreed that the protein molecule must be split into smaller molecules before it can be acted upon by the intracellular enzymes of oxidation and reduction. Protein, therefore, is hydrolyzed by extracellular proteases comprising proteinases and peptidases (Grassmann and Schneider, 1936) which attack their substrates by opening the peptid linkage (CO–NH and CO–N). The former attack true proteins and function at different pH levels; the latter attack peptids. Strict differentiation of the two types is difficult. Intracellular dissimilation of proteins is essentially that of the amino acids after extracellular hydrolysis of the non-diffusible protein molecule. Our knowledge of the kinetics of amino acid breakdown is fragmentary and little is known regarding the intermediary metabolism. At present the best we can do is to discuss the overall conversion of amino acids into final products, omitting in large measure reference to the intermediate products, catalysts and coenzymes involved.

The overall reactions of certain of the dissimilative processes brought about by bacteria acting on amino acids may be classified; very little is known of the assimilative changes.

The changes brought about by microorganisms acting on amino acids may be oxybiontic or anoxybiontic. Changes occurring anoxybiontically involve decarboxylation, deamination, hydrogen transfer from donator to an acceptor or liberation of gaseous hydrogen. Oxybiontically we have the same changes and in addition the formation of water or peroxide as the result of oxygen acting as a hydrogen acceptor. The changes brought about by microorganisms in amino acids may be illustrated.

1. Hydrolytic deamination:

$$RCHNH_2COOH + HOH \rightarrow RCHOHCOOH + NH_3$$
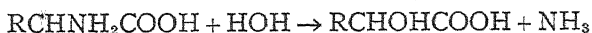
Arai (1921) reports that *B. proteus* hydrolytically deaminates *l*-(–)leucine to form *d*-leucic acid; Schmidt, Peterson and Fred (1924) report *l*-leucic acid probably from leucine in maize protein. *l*-(+) aspartic acid by *E. coli* (Woolf, 1929), *l*-(–)histidine by *B. proteus* (Hirai, 1919), *l*-(–) phenylalanine by *B. subtilis* and

B. proteus (Sasaki and Otsuka, 1921) have been claimed to undergo hydrolytic deamination. No direct evidence, however, was offered in these cases that the hydroxy acid was a product of hydrolytic deamination. Virtanen and Erkama (1938) claim direct evidence of a hydrolytic deaminase in *Pseudomonas fluorescens* forming malic acid directly from aspartic acid, $COOH \cdot CH_2 \cdot CHNH_2COOH + H_2O \rightarrow COOH \cdot CH_2 \cdot CHOH \cdot COOH + NH_3$.

The deamination may be accompanied by decarboxylation of the hydroxy acid to form a primary alcohol, $RCHOHCOOH \rightarrow CO_2 + RCH_2OH$.

Hydrolytic deamination followed by decarboxylation to yield a primary alcohol with one less C atom occurs commonly among the yeasts, torula, *Monilia* and other fungi.

2. Decarboxylation to yield an amine with one less carbon atom:

$$R \cdot CHNH_2COOH \rightarrow RCH_2NH_2 + CO_2$$
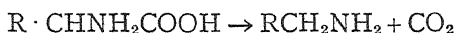
Decarboxylation of amino acids by bacteria is of frequent occurrence. *B. fluorescens-liquefaciens* and *Streptococcus longus* (Emmerling, 1897, Emmerling and Reiser, 1902) to yield methylamine from glycine, *iso*-amylamine from l-($-$) leucine by *B. proteus* and *B. subtilis* are examples. In the case of $l(+)$ aspartic and l-($+$) glutamic acid, containing two carboxyl groups, only one is decarboxylated to yield the corresponding β-mono-carboxylic acid thus.

$$COOH \cdot CH_2CHNH_2COOH \rightarrow CO_2 + CH_2(NH_2)CH_2COOH$$
aspartic acid   β-alanine
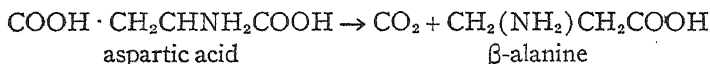
Gale (1940*a*) showed that washed suspensions of *E. coli* decarboxylate $l(+)$-arginine, $l(+)$-lysine, $l(+)$-ornithine, $l(-)$-histidine and $l(+)$-glutamic acids to agmatine, cadaverine, putrescine, histamine and γ-aminobutyric acids respectively. Of the fourteen strains of coliform bacteria investigated, twelve decarboxylated arginine, histidine and ornithine, thirteen lysine and nine glutamic acid. Carboxylase activity in the organisms depended on the pH of growth; organisms grown at pH 7.0 showed little activity which is increased 20- to 100-fold by growth at pH 5.0. Washed suspensions of *Streptococcus faecalis* (Gale, 1940*b*) decarboxylate $l(-)$-tyrosine to form tyramine. The enzyme was highly specific for tyrosine; no other amino acid was attacked.

3. Hydrogenation to yield an amine and formic acid:

$$R \cdot CHNH_2COOH \xrightarrow{+2H} RCH_2NH_2 + HCOOH$$
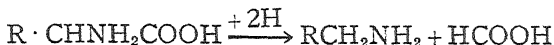
4. Dehydrogenation and hydrolytic deamination (oxidative deamination),

$$R \cdot CH \cdot NH_2COOH + Acc \longrightarrow RC:NHCOOH + Acc\,H_2$$
$$RC:NHCOOH + H_2O \longrightarrow RCOCOOH + NH_3$$
and decarboxylation
$$RCOCOOH + HOH + Acc \rightarrow CO_2 + RCOOH + Acc\text{-}H_2$$
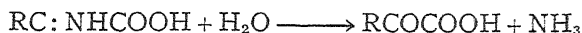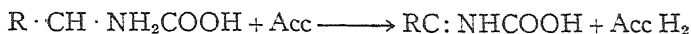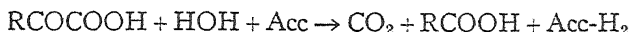
Krebs (1932, 1933a, 1933b) showed the following reaction for kidney tissue in the case of glutamic acid.

$$COOH \cdot CH_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH + H_2O \xrightarrow{+O}$$
$$\text{glutamic acid}$$
$$COOH \cdot CH_2 \cdot CH_2COCOOH + NH_3 + H_2O$$
$$\alpha\text{-keto glutaric acid}$$

$$COOHCH_2CH_2COCOOH + HOH \rightarrow$$
$$COOHCH_2CH_2COOH + 2\,H + CO_2$$
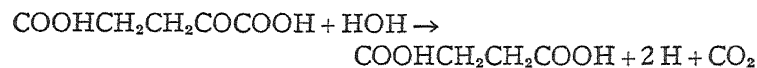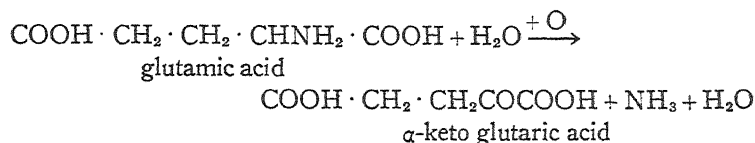
Needham (1927, 1930) postulated and Braunstein and Kritzmann (1937–1938) experimentally proved that succinic acid may also be formed anaerobically by muscle in a similar manner when pyruvic acid replaces O_2 as a H_2-acceptor.

Stephenson and Gale (1937) showed that washed suspensions of *E. coli* deaminate glycine, *dl*-alanine and *l*(+) glutamic acid aerobically but not anaerobically. Janke and Tayenthol (1937) by the use of glycine showed this deamination by *E. coli, Pseudomonas fluorescens, Proteus vulgaris* and *Bacillus mycoides* aerobically but not anaerobically unless an oxidizing agent was present, such as *m*-dinitrobenzene. The reaction may be represented by the equation: $NH_2CH_2COOH + \frac{1}{2}O_2 = CHOCOOH + NH_3$. Aubel and Egami (1936) show oxidative deamination of *dl*-alanine by a soil organism. The reaction did occur anaerobically in the presence of nitrate. They believed that dehydrogenation first occurred followed by hydrolysis of the resulting imino acid.

With an *l*(+)-glutamic acid deaminase preparation extracted from *E. coli*, Adler et al. (1938) showed reduction of methylene blue in the presence of glutamic acid. Coenzyme II was required. The studies of Adler *et al.* have led to the general scheme:

Glutamic acid + coenzyme II $\rightleftharpoons$ Iminoglutaric acid + dihydro-coenzyme II

Iminoglutaric acid + H_2O $\rightleftharpoons$ Ketoglutaric + NH_3

Dihydro-coenzyme II + $\frac{1}{2}$ O_2 = Coenzyme II + H_2O

They believe the reversible reaction is important in amino acid synthesis.

Klein (1940) with washed suspensions of *Hemophilus parainfluenzae* showed oxidation of $l(-)$-aspartic and $l(+)$ glutamic acids with the liberation of ammonia and CO_2 and the formation of acetic acid.   Coenzyme I or II is required.

Woods and Clifton (1937) found *Clostridium tetanomorphum* to break down certain amino acids with the liberation of gaseous H_2, *i.e.,* no hydrogen acceptor was required.

5. Hydrogenation and deamination to form the fatty acid:

$$RCHNH_2COOH + H_2 \rightarrow RCH_2COOH + NH_3$$
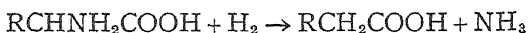
This type of attack occurs among bacteria.   The formation of succinic acid from aspartic by *E. coli* (Harden, 1901), by *B. proteus* (Nawiasky, 1908) and the propionic acid bacteria (van Niel, 1928), glutaric or succinic acid from glutamic acid (Ackermann and Mey, 1906) or indole propionic acid from tryptophane by *E. coli* (Hopkins and Cole, 1901–1902) are examples.

Quastel and Woolf (1926) by the use of *l*-aspartic acid and suspensions of *E. coli,* showed that in the presence of an inhibitor such as toluene, the reaction did not go to completion and resulted in the formation of fumaric acid, $COOH \cdot CH_2 \cdot CHNH_2COOH \rightleftharpoons COOHCH : CHCOOH + NH_3$.   Virtanen and Tarnanen (1932) obtained aspartase from *Pseudomonas fluorescens* and thus proved the existence of a desaturation deaminase.

Cook and Woolf (1928) found strict aerobes and anaerobes did not bring about the following reaction, $COOH \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH \rightarrow COOH \cdot CH : CH \cdot COOH + NH_3$, and inasmuch as succinic acid was formed, the reaction must be accepted to be a hydrogenation of the aspartic with deamination: $COOH \cdot CH_2 \cdot CHNH_2COOH + 2H \rightarrow COOH \cdot CH_2 \cdot CH_2 \cdot COOH + NH_3$.

6. Hydrogenation, deamination, and decarboxylation:

$$RCHNH_2COOH + 2H \rightarrow RCH_3 + NH_3 + CO_2$$
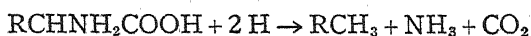
Methane might be formed in this manner from glycine by anaerobes.

$$CH_2NH_2COOH + 2 H \rightarrow CH_4 + NH_3 + CO_2$$
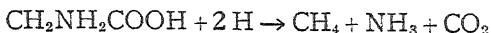
Propionic acid is reported from aspartic acid by Borchardt (1909) with a mixed culture, and by Brasch (1909), for *B. putrificus*.

7. Dehydrogenation, deamination and decarboxylation:

$$RCHNH_2COOH + 2 HOH + 2 Acc \rightarrow$$
$$RCOOH + NH_3 + CO_2 + 2 \ Acc\text{-}H_2$$
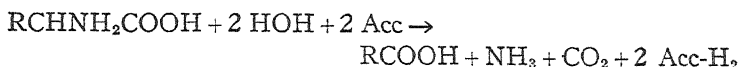
The type of bacterial attack on amino acids depends on a number of factors: (*a*) the organism involved, (*b*) the amino acid attacked and (*c*) environmental conditions.

Considering first the variation of the type of breakdown with the organism; anaerobes must, of course, bring about the dissimilation of amino acids without the aid of free oxygen as a hydrogen acceptor. They must, therefore, have present (*a*) a system providing sufficient energy, (*b*) suitable hydrogen acceptors, (*c*) necessary accessory factors. It is apparent that bacteria growing anaerobically will not convert their substrate completely into CO_2 and water as do the aerobes but must necessarily have as their final products, the ultimate reduced acceptors of hydrogen. Thus H_2O is the final reduced acceptor of an aerobe corresponding to butyl alcohol in the butyl alcohol fermentation where butyric acid is the acceptor, or ethyl alcohol in the case of ethyl alcohol production where acetaldehyde is the acceptor. In the case of protein metabolism, however, amino acids function as acceptors; thus the propionic acid bacteria reduce and deaminate aspartic acid to form succinic acid:

$$COOHCH_2CHNH_2COOH + 2 H \rightarrow$$
$$COOH \cdot CH_2 \cdot CH_2COOH + NH_3$$
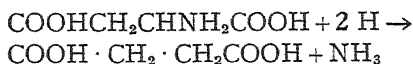
In this case the aspartic acid, when a constituent of a normal glucose medium, competes with hydrogen acceptors derived from glucose breakdown, probably lactic acid which is reduced to propionic acid.

Secondly, the amino acid attacked determines the type of breakdown and the products. Hanke and Koessler (1924) showed quite

definitely that ability to decarboxylate tyrosine did not indicate ability to do the same to histidine.

Thirdly, bacterial attack on amino acids is determined by environmental conditions, *e.g.*, pH, constituents of the medium, aerobic or anaerobic conditions. The effect of environment may be illustrated by the work of Sasaki (1914) who studied the action of *Proteus vulgaris* and *Escherichia coli* on tyrosine. The products with both organisms depended on the constituents of the medium. When lactose replaced phosphate, *p*-hydroxyphenylethylamine was formed by both organisms; otherwise, *p*-hydroxyphenyllactic acid was formed.

The reactions of bacteria on the amino acids have been presented as though they occur in the cell unrelated to carbohydrate metabolism, show only dissimilative abilities of the organisms and occur largely without the aid of coenzymes. Such is not the case. First, although relatively little is known, it is clear that the carbon and nitrogen metabolisms of bacteria are intimately related into one unified whole. Braunstein and Kritzmann (1937, 1937–1938) have shown transamination in animal tissue in which the amino group of either of the natural dicarboxylic amino acids is transferred to a monocarboxylic keto acid, thus

$CHNH_2 \cdot COOH$

$\qquad + R \cdot CO \cdot COOH \rightleftharpoons$

CH_2COOH

$\qquad\qquad CO\quad COOH$

$\qquad\qquad\qquad\qquad + R \cdot CHNH_2 \cdot COOH$

$\qquad\qquad CH_2 \cdot COOH$
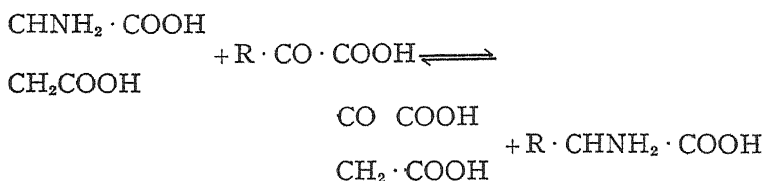
Furthermore, Virtanen and Laine (1938, 1939) claim that the symbiotic nitrogen fixing *Rhizobium* can fix atmospheric nitrogen with the formation of hydroxylamine which combines with oxaloacetic acid, a product of carbohydrate dissimilation, to form aspartic acid. Virtanen and Laine (1938) also claim to have shown the formation of alanine by the transfer of the amino group from aspartic acid to pyruvic acid in the presence of the host plant.

Secondly, with reference to assimilation, the stage has not been reached where an attack can be made with accuracy and understanding. A few of the reactions described with amino acids are known to be reversible and the work of Braunstein and Kritzmann

and Virtanen and Laine may lead the way to a clearer understanding of this important phase of metabolism.

Thirdly, the first knowledge is being gained regarding the coenzymes required in protein and amino acid metabolism.

The enzymes concerned in the primary utilization of amino acids by bacteria is reviewed by Gale (1940c).

HETEROTROPHIC ASSIMILATION OF CARBON DIOXIDE

Wood and Werkman presented the first experimental evidence in 1935 that heterotrophic non-photosynthetic bacteria assimilate carbon dioxide. Their proposals necessitated acceptance of a new principle in the metabolism of such bacteria.

Assimilation of CO_2 is here defined as its utilization to form a compound in which a carbon to carbon linkage is created; the definition does not include simple reduction of CO_2 to form, *e.g.*, formic acid, as shown to occur by Woods with *Escherichia coli* (1936).

Photosynthetic and chemosynthetic organisms have in the past been considered the only forms utilizing CO_2 as a metabolite. There was some evidence, however, that CO_2 played an active rôle in the metabolism of organisms, inasmuch as it was shown by a number of investigators (Smith, 1924; Rockwell and Highberger, 1927) that a minimum concentration of CO_2 is necessary for the growth of many bacteria. There was, however, no indication of the function of the CO_2 in metabolism. Wood and Werkman (1935, 1936) using the propionic acid bacteria, showed that in the fermentation of glycerol, there is a net uptake of CO_2, and that the carbon of this CO_2 is accounted for in the products, acetic, propionic and succinic acids. It, thus, became clear that a non-photosynthetic, typical heterotrophic cell was able to assimilate CO_2. Shortly afterwards other investigators showed that CO_2 was reactive in the metabolism of heterotrophic bacteria. Woods (1936) demonstrated that *E. coli* reduces CO_2 to formic acid with gaseous hydrogen; also Barker (1936) proved that CO_2 is a hydrogen acceptor, being converted to methane by the methane bacteria.

Considerable misunderstanding has arisen, however, concerning the relative significance of the above investigations. Obviously "fixation" of CO_2 occurred by two distinct types of reactions in the respective experiments: (1) reduction with no creation of carbon to carbon linkages, and (2) creation of the carbon to carbon link-

age. In the former, CO_2 is functioning only as a hydrogen acceptor; the essential requirement of the latter scheme is the creation of a carbon to carbon linkage. Much confusion has arisen as a result of the failure to differentiate the two types of reaction. The Wood and Werkman reaction is of fundamental importance not only because it demonstrates heterotrophic assimilation of CO_2, but also because it bears indications of leading the way to the elucidation of photosynthetic as well as chemosynthetic assimilation of CO_2 in plants, and has already led to new concepts of CO_2 utilization in animal tissues. The proposals of Wood and Werkman were not at first accepted, although confirmation by Phelps *et al.* (1939) and Carson and Ruben (1940) removed any remaining doubt.

Weiringa (1936, 1940) showed reduction of CO_2 to acetic acid by hydrogen in the presence of an extract of mud with a facultative organism, facultative in the sense that it grew, *i.e.*, utilized CO_2 as the only source of carbon, but showed heterotrophism in that it also used glucose as a source of carbon. Weiringa's demonstration is not that of a typically heterotrophic utilization of CO_2.

The first hint with regard to the mechanism of heterotrophic CO_2 assimilation came from Elsden (1938) who showed that the rate of succinic acid formation by *E. coli* from a number of substrates is a function of the concentration of CO_2 in the medium. No direct evidence was obtained for fixation of CO_2 but it was suggested that CO_2 might be involved in the synthesis of succinic acid. Independently Wood and Werkman (1938) were able to present rather direct evidence of the relationship of CO_2 fixation to succinic acid formation. They demonstrated in the fermentation of glycerol by propionic acid bacteria that there was an equimolar relationship between the succinic acid formed and the CO_2 fixed. In view of their results, the proposal was made that succinic acid was synthesized by union of 3-carbon and 1-carbon compounds, and CO_2 fixed by this mechanism. Pyruvic acid was suggested as the probable intermediary 3-carbon compound. Wood and Werkman (1940a) showed that CO_2 is fixed by propionic acid bacteria fermenting a number of substrates other than glycerol. Malonate, azide, arsenite, cyanide and pyrophosphate were found to have no influence on CO_2-fixation, whereas NaF and iodoacetate were inhibitory. In 1940 the same authors showed that inhibition of fixation of CO_2 by NaF caused an equivalent decrease in the succinic

acid yield, which was considered as further evidence that CO_2 was fixed by union with a 3-carbon compound.

It was not until the isotopes of carbon (radioactive carbon, atomic weight eleven, and the stable isotope, atomic weight thirteen) became available for use as tracers of fixed carbon dioxide that unquestionable proof of fixation of CO_2 to form succinic acid was obtained. Carson and Ruben (1940), using radioactive carbon, demonstrated conclusively that CO_2 is fixed in succinic acid and propionic acid in the fermentation of glycerol by propionic acid bacteria. Wood *et al.* (1940, 1941*a*) independently obtained similar results with the stable isotope of carbon, C^{13}. Furthermore, they showed that carbon dioxide is fixed in the fermentations of galactose, pyruvic acid, and citric acid by coliform bacteria, and that in this case the fixed carbon dioxide occurs solely in the succinic and formic acids. The results with the coliform bacteria fit into the picture of fixation of CO_2 by union of 3- and 1-carbon compounds, since aside from formic acid, which undoubtedly was formed by reduction of CO_2 with hydrogen (Woods, 1936), the 4-carbon compound succinic acid was the only product containing fixed carbon. The results from the propionic acid fermentation are not so easily explained since propionic acid likewise contained fixed CO_2.

If fixation occurs by the union of 3-carbon and 1-carbon compounds, the fixed carbon in the succinic acid will be located in the carboxyl group. The following reactions indicate the probable mechanism for such a synthesis; the carbon dioxide has been labeled with the carbon isotope (C^{13}).

$$C^{13}O_2 + HCH_2 \cdot CO \cdot COOH = C^{13}OOH \cdot CH_2 \cdot CO \cdot COOH$$

$$C^{13}OOH \cdot CH_2 \cdot CO \cdot COOH + 4H =$$
$$C^{13}OOH \cdot CH_2 \cdot CH_2 \cdot COOH + H_2O$$
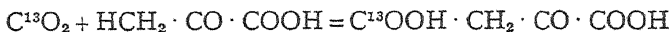
Wood *et al.* (1940, 1941*b*) have provided proof of this location of the fixed carbon of succinic acid. They decarboxylated biologically formed succinic acid, containing C^{13} from fixed $C^{13}O_2$, by two methods. First,

$$COOH \cdot CH_2 \cdot CH_2 \cdot COOH \xrightarrow[\text{dehydrogenase}]{\text{succinic}}$$
$$COOH \cdot CH : CH \cdot COOH$$
$$\text{fumaric acid}$$

$$\xrightleftharpoons[]{\text{fumarase}} \quad COOH \cdot CH_2 \cdot CHOH \cdot COOH$$

$$COOH \cdot CH_2 \cdot CHOH \cdot COOH \xrightarrow{KMnO_4}$$

$$\begin{aligned} \text{malic acid} \\ (COOH \cdot CH_2 \cdot CHO) + CO_2 \\ \downarrow \\ CO_2 + CH_3CHO \end{aligned}$$
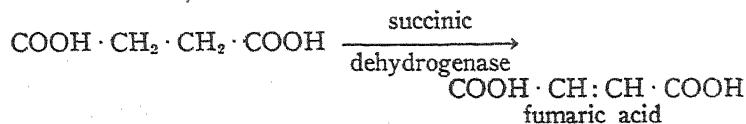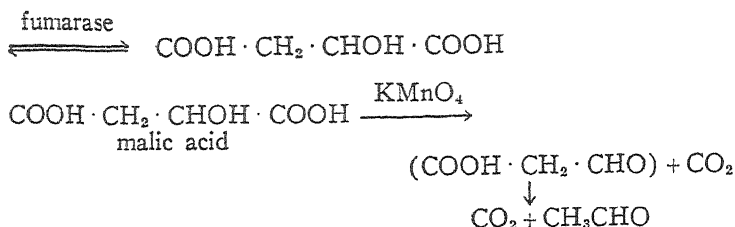
It is apparent that the two carbon dioxide molecules are formed from the carboxyl groups, and acetaldehyde from the methylene groups of the succinic acid. By determination of the C^{13} content of the carbon dioxide and acetaldehyde the location of the fixed C^{13} in the succinic acid was established.

In the second method the malic acid was prepared as in the previous method.

$$COOH \cdot CH_2 \cdot CHOH \cdot COOH \xrightarrow[\text{dehydrogenase}]{\text{malic}}$$

$$\begin{aligned} \text{malic acid} \\ COOH \cdot CH_2 \cdot CO \cdot COOH \\ \text{oxaloacetic acid} \end{aligned}$$

$$COOH \cdot CH_2 \cdot CO \cdot COOH + H\overset{H}{\overset{\cdot}{N}} \cdot C_6H_5 \xrightarrow[\text{acid}]{\text{citric}}$$

$$\begin{aligned} (COOH \cdot CH_2 \cdot CO \cdot CO \cdot \overset{H}{N} \cdot C_6H_5) \\ \downarrow \\ CO_2 + CH_3 \cdot CO \cdot CO \cdot \overset{H}{N} \cdot C_6H_5 \end{aligned}$$
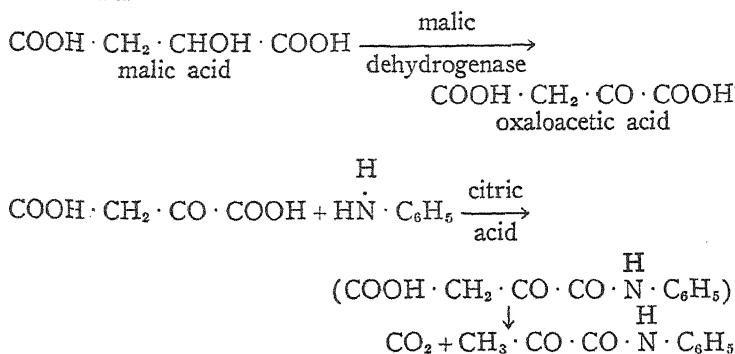
The carbon dioxide thus originates from the carboxyl groups of succinic acid. Determination of the C^{13} in the carbon dioxide, therefore, provided a measure of the fixed carbon in the carboxyl group.

The data from both methods showed that the methylene carbons of succinic acid contained only the natural complement of C^{13}. The carboxyl carbons, on the contrary, contained a concentration of C^{13} well above the natural, and in amounts sufficient to account for all the fixed C^{13} in the succinic acid. It is thus evident that the fixed carbon dioxide is exclusively in the carboxyl groups of the acid. This is in agreement with the proposal that carbon dioxide is fixed by union with pyruvic acid.

The above results were obtained with succinic acid formed in the

fermentation of galactose and pyruvate by *E. coli,* and glycerol by propionic acid bacteria. The fact that *E. coli* forms succinic acid from pyruvic acid with fixation of carbon dioxide in the carboxyl groups lends weight to the suggested intermediary rôle of pyruvic acid in carbon dioxide assimilation.

It is not possible to prove by degradation of a symmetrical dicarboxylic acid that the fixed C^{13} is in only one of the carboxyl groups, a requirement of the proposed reaction of pyruvic acid with CO_2. However, Wood *et al.* (1941*a*), on the basis of quantitative data, did offer indirect proof that the requirement is met. They showed that the C^{13} content of the succinic acid determined experimentally was in agreement with the calculated values on the basis that $C^{13}O_2$ is fixed in only one carboxyl of any one succinic acid molecule. On the contrary, values calculated on the basis that $C^{13}O_2$ is in both carboxyl groups of the succinic acid molecule, were much higher than those observed.

The question arises whether the same mechanism of fixation is involved in the formation of propionic acid, possibly decarboxylation of succinic acid (or some other dicarboxylic acid) occurs to yield propionic acid and carbon dioxide (Wood, Stone, and Werkman, 1937). Accordingly the fixed carbon in propionic acid would be in the carboxyl group. Therefore, the report of Carson *et al.* (1940) of fixed carbon dioxide occurring in the α- and β-carbons, as well as in the carboxyl carbon of propionic acid, is interesting. They degraded propionic acid containing fixed radioactive carbon. The acid was obtained by fermenting glycerol with propionic acid bacteria under an atmosphere containing radioactive CO_2. Degradation was carried out by heating dry barium propionate to 350° C. and by alkaline permanganate oxidation. The first conversion yields diethyl ketone and barium carbonate the second, oxalate and carbonate. It was assumed, particularly in the first reaction, that the carbonate arose only from the carboxyl group of the propionic acid. The radioactivity of the carbonates was by no means sufficient to indicate that the fixed carbon of the molecule was exclusively in the carboxyl group. The distribution of the C^{11} between the diethyl ketone, oxalate and carbonates was more nearly in agreement with a distribution of the fixed carbon evenly along the chain. In accordance, these investigators proposed that fixation of CO_2 by propionic acid bacteria occurred in the α and β positions as well as in the carboxyl groups.

This proposal implies that in addition to the mechanism of union of 3- and 1-carbon compounds, the propionic acid bacteria have at least another method of fixing carbon dioxide. Furthermore, since it is suggested that the fixed carbon dioxide is distributed among the three carbons of the propionic acid, it is evident that the acid may have been completely synthesized from 1-carbon compounds. While such a synthesis is true for autotrophic forms, there is at present no evidence that typical heterotrophic organisms break down the substrate to 1-carbon compounds, and then synthesize the final products from these. If such is the case, it will necessitate a complete revision of schemes of heterotrophic fermentation.

Wood *et al.* (1941*c*) have, therefore, reinvestigated the problem, using propionic acid from the fermentation of glycerol containing fixed C^{13}. Contrary to the report of Carson *et al.*, they have found the fixed carbon exclusively in the carboxyl group of the acid. The degradation was accomplished by the following reactions:

$$CH_3 \cdot CH_2 \cdot COOH \xrightarrow{\text{P and Br, then } H_2O} CH_3 \cdot CHBr \cdot COOH$$

$$CH_3 \cdot CHBr \cdot COOH \xrightarrow{\text{AgOH}} CH_3 \cdot CHOH \cdot COOH$$

$$CH_3 \cdot CHOH \cdot COOH \xrightarrow{\text{KMnO}_4} CH_3CHO + CO_2$$

$$CH_3CHO \xrightarrow{I_2 \text{ (alkali)}} CHI_3 + HCOONa$$
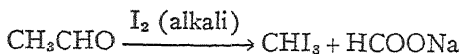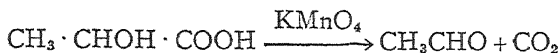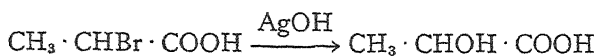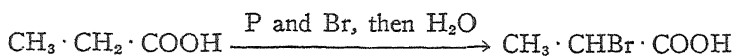
The above degradation yields CO_2 from the carboxyl group, and acetaldehyde from the α- and β-carbons of the propionic acid. The acetaldehyde was degraded in turn to give formate containing the α-carbon, and iodoform containing the β-carbon. Of these compounds only CO_2, corresponding to the carboxyl carbon, contained fixed C^{13}. The quantity of C^{13} in the CO_2 was sufficient to account for all the C^{13} fixed in the propionic acid. It is evident that these results are in agreement with the concept that all the carbon dioxide is fixed through synthesis involving 3-carbon and 1-carbon compounds. That the stepwise degradation used by Wood *et al.* was reliable is evident, for the CO_2 and acetaldehyde were formed in equal molar amounts, proof that CO_2 was formed only from the carboxyl group, yet the C^{13} was accounted for quantitatively in this CO_2.

Wood *et al* (1941*a*) have shown that the quantitative relationships of the C^{13} in the products of fermentation of glycerol by propionic acid bacteria, are in agreement with the proposal that propionic acid is formed exclusively by decarboxylation of a symmetrical dicarboxylic acid containing fixed C^{13} in one carboxyl group.   In such a decarboxylation the chances are equal of splitting off $C^{12}O_2$ from the carboxyl originating from the glycerol ($C^{12}O_2$) and $C^{13}O_2$ from the carboxyl formed from fixed carbon.   Therefore, requirements of this reaction are that the concentration of C^{13} in the carboxyl group of propionic acid be equal to the average concentration of the C^{13} in the two carboxyl groups of succinic acid and that the total $C^{13}O_2$ of the medium (gas and bicarbonate) be diluted by $C^{12}O_2$ in amounts equivalent to one-half the propionic acid. For example, if 100 mM of succinic acid containing fixed $C^{13}O_2$ in one carboxyl are decarboxylated to 100 mM of propionic acid, 50 mM of $C^{13}O_2$ and 50 mM of $C^{12}O_2$ will be formed.   The experimental results were in agreement with the theoretical values.   It now seems probable that propionic acid may be formed in general in this fermentation by the previously unexpected mechanism of union of a 3-carbon compound and CO_2 with reduction and subsequent cleavage to propionic acid.   The liberated CO_2 is then used again in a similar cycle of events.   Of the several schemes suggested in the past for formation of propionic acid, none has received support in spite of efforts by a number of investigators.

The question arises whether the assimilation of CO_2 through synthesis with 3- and 1-carbon compounds, is limited to bacterial fermentations.   Recently, there has been strong evidence that this reaction plays a part in the metabolism of higher animals, particularly in the Krebs citric acid cycle (Krebs and Johnson, 1937; Krebs and Eggleston, 1940*a*).   The Krebs cycle is a rather complicated series of reactions which accounts for the mechanism of aerobic oxidation of pyruvic acid to CO_2 and water.   Its essential features are first the union of oxaloacetic acid and pyruvic acid to form a seven carbon compound which immediately is decarboxylated and oxidized to citric acid.[1]   The citric acid is in turn oxi-

[1] It is possible that citric acid is formed in the dissimilation of glucose by molds through a similar mechanism.   Accordingly, the glucose on 3-carbon cleavage yields pyruvic acid; then one of the molecules of pyruvic acid is converted to oxaloacetic acid by union with CO_2.   A molecule of oxaloacetic acid and pyruvic acid then unite, and the resulting compound is converted by decarboxylation and oxidation to CO_2 and citric acid.   The CO_2 can then function again in the cycle.

dized and decarboxylated first to α-ketoglutaric acid, then to succinic acid which is in turn converted to oxaloacetic acid. The net result is oxidation of the pyruvic acid to CO_2 with regeneration of oxaloacetic acid which may again serve in the cycle. Wood and Werkman (1938) suggested that this cycle involved CO_2 utilization. Since its inception the writers have been interested in the development of this cycle, for obviously it involves reactions which lead to synthesis or, on the other hand, decarboxylation. Decarboxylations are of interest because the reverse reaction may occur, *i.e.,* carboxylation and thus fixation of CO_2. Reversing the citric acid cycle would thus yield a synthesis of pyruvic acid from CO_2, *i.e.,* three molecules of CO_2 might unite stepwise with oxaloacetic acid to form ultimately a seven carbon compound which would then be split to pyruvic acid and oxaloacetic acid. The latter compound would function again in the cycle.

The investigations of Evans (1940) showed that pigeon liver with pyruvic acid as the only substrate could carry on the Krebs cycle. Since the cycle involves the combination of oxaloacetic acid and pyruvic acid, it appeared very probable that the synthesis of oxaloacetic acid from pyruvic acid and CO_2 was taking place. Evans and Slotin (1940) soon provided the experimental evidence that CO_2 fixation occurs in the Krebs cycle. They showed by using CO_2 containing radioactive carbon that CO_2 is fixed in α-ketoglutaric acid formed by pigeon liver from pyruvic acid. Although this does not constitute conclusive proof that the CO_2 in the α-ketoglutaric acid was originally fixed by 3-carbon and 1-carbon addition, it is a reasonable assumption.[2]

Krebs and Eggleston (1940*b*) have presented indirect evidence for the synthesis of oxaloacetic acid from pyruvic acid and CO_2. In addition they are of the opinion that thiamin is essential in this reaction. The principal point advanced is that the rate of pyruvate oxidation in pigeon liver depends upon the concentration of CO_2

[2] Note added July 9, 1941. Recently Wood *et al.,* 1941*d*, and Evans, 1941, have shown that the carbon fixed by pigeon liver in α-ketoglutarate is located exclusively in the carboxyl group adjacent to the carbonyl group. This fact necessitates a revision of our ideas on the Krebs cycle, for it is apparent that if citrate was a precursor of α-ketoglutarate, the fixed carbon would be in both carboxyl groups of the glutarate (*cf.* Krebs, 1941). There is evidence, however, that the CO_2 is fixed by 3- and 1-carbon addition by pigeon liver for Wood *et al.,* 1941*e* have found fixed carbon in the carboxyl group of malate, fumarate, and succinate formed from pyruvate. Lactate formed in these dissimilations, likewise, contained fixed carbon in the carboxyl group. The mechanism of the latter fixation is uncertain.

and bicarbonate. Their opinion is that the CO_2 is used in the synthesis of oxaloacetic acid from pyruvic acid which then serves in the Krebs cycle for the further breakdown of the pyruvate. The explanation seems reasonable, particularly in view of the results of Evans and Slotin. The evidence for the proposed function of thiamin is based on the observation that thiamin on addition to muscle and liver suspensions from thiamin-deficient pigeons causes an increase in pyruvate dissimilation by liver but not by muscle. The vitamin was presumed to take part in a reaction present in liver but absent in the muscle. The reaction, pyruvic acid + CO_2 → oxaloacetate, is believed by Krebs and Eggleston to occur in liver but not in muscle. They, therefore, suggest the vitamin acts in this conversion.

The observations of Smyth (1940) provide more convincing and direct evidence of such a catalysis. With thiamin-deficient staphylococci the dismutation of pyruvic acid could be accelerated either by thiamin or oxaloacetate (without thiamin). In the past it has been considered that thiamin is absolutely essential for the dissimilation of pyruvic acid. Krebs, Eggleston and Smyth now suggest that thiamin acts only in the formation of oxaloacetate and this compound serves as a hydrogen carrier; therefore, if oxaloacetate is added, thiamin is not required.

The results of Krampitz and Werkman (unpublished results) are not in accord with these results. These investigators have been able to demonstrate that there is a heat-labile enzyme present in *Micrococcus lysodeikticus* which catalyzes the decarboxylation of oxaloacetic acid. Neither thiamin nor cocarboxylase is required for this decarboxylation but magnesium ions are essential. Preparations of these bacteria, free of cocarboxylase and Mg^{++}, will not decarboxylate or oxidize either oxaloacetic acid or pyruvic acid. When Mg^{++} is added, the decarboxylation of oxaloacetic acid proceeds both aerobically and anaerobically but the resulting pyruvic acid is not oxidized. When both Mg^{++} and cocarboxylase or thiamin are added, the pyruvic acid is converted to CO_2 and acetic acid with O_2 uptake. The addition of oxaloacetic acid to pyruvic acid dissimilation in no way influences the oxygen uptake or CO_2 liberation from pyruvic acid. It is, therefore, evident that the function of cocarboxylase in the oxidation of pyruvic acid by these bacteria is not to catalyze the formation of oxaloacetate. Assum-

ing that the same enzyme catalyzes the synthesis as well as the breakdown of oxaloacetate, the enzyme of Krampitz and Werkman does not need cocarboxylase to synthesize oxaloacetic acid from pyruvic acid and CO_2.

An explanation of the results obtained by Smyth is not at hand but it is obvious that there may be other explanations.

Krebs and Eggleston are of the opinion that there is a close analogy between the decarboxylation of pyruvic acid and oxaloacetic acid and cite decarboxylation of both acids by aniline as evidence. There is no convincing evidence, however, that these chemical decarboxylations with aniline occur by similar mechanisms. On the other hand, there is reason to think the reactions may not be similar; in pyruvic acid the carboxyl is adjacent to a carbonyl group and in oxaloacetic acid the carboxyl involved is adjacent to a methylene group and the compound is highly enolized.

That mammalian tissue, as well as other heterotrophic non-photosynthetic cells such as yeast, ground barley roots and *E. coli,* fix CO_2 has been shown by Ruben and Kamen (1940a), using $C^{11}O_2$. Quantitatively much less CO_2 was fixed than in the experiments described above; also, a different mechanism may be involved. This assimilation is inhibited by HCN, and it is believed to have a function in synthesizing a specific oxidizing agent. Hes (1938) has reported that a trace of CO_2 is essential in the reduction of methylene blue by bacteria. The nature of this function of CO_2 has never been explained, but CO_2 may be necessary for the synthesis of 4-carbon dicarboxylic acids which then serve as hydrogen carriers or participate in the Krebs cycle. Attempts by Ruben and Kamen to identify the radioactive compounds were unsuccessful. The cells were boiled with dilute acid for one minute; this cell-free aqueous extract contained 90% of the reduced C^{11}. The cell-free medium contained only 1% of the C^{11} if acid boiling treatment was omitted. The C^{11} compound was not pyruvic acid. Salts of Ba^{++}, precipitated in 80% alcohol, were very active. Only 3% of the C^{11} was obtained as $BaCO_3$ by decarboxylation of the Ba salt at 250° C.

Barker, Ruben and Kamen (1940) and Barker (1941) have shown that CO_2 is used as a hydrogen acceptor by the methane bacteria, resulting in its reduction to methane. They also claim that some of the CO_2 is assimilated into organic constituents of the cells. Although CO_2 assimilation to form protoplasm is probably

of considerable general occurrence among heterotrophs, the evidence offered hardly constitutes proof. As nearly as can be determined from the description of the experimental procedure, Barker *et al.* removed the volatile material from the fermentation by steam distillation and then evaporated the residue to dryness. The dry residue was called cell material. It is quite clear that any nonvolatile material, whether part of the cell or not, would be obtained by this procedure. If it is desired to show that an organism assimilates CO_2 in its protoplasm, it would seem desirable to use proliferating rather than non-proliferating cells. In this case the organism would be forming its protoplasm. In all experiments so far reported in which the isotopes of carbon have been used, non-proliferating cells were employed. The question may be raised as to what constitutes cell protoplasm. If the assimilated CO_2 is washed out with dilute acid, is it to be considered protoplasm or may it not be adsorbed material?

Barker, Ruben and Beck (1940) have made an interesting contribution concerning the synthesis of acetic acid from CO_2 by *Clostridium acidi-urici.* This organism ferments uric acid, xanthine, hypoxanthine and other purines under anaerobic conditions, yielding cell material, ammonia, CO_2 and acetic acid. With hypoxanthine more than one mole of acetic acid was obtained per mole of substrate. Obviously a synthesis was involved. With radioactive CO_2 it was possible to show that this synthesis of acetic acid involved assimilation of CO_2. Of even greater interest is the apparent proof that the fixed CO_2 is in both the methyl and carboxyl groups of the acetic acid. The barium salt of the acid was degraded by heating to 450°–500° C. for 50 minutes. The resulting acetone was subjected to the iodoform reaction, and the iodoform, which presumably arises from carbon originally in the methyl group of the acetic acid, was investigated. The $BaCO_3$ formed during the heating was likewise tested and considered as carboxyl carbon. Sixty-seven per cent of the fixed carbon was found in the methyl group and 33% in the carboxyl group. Barker *et al.* suggest that the fixed C^{11} in the carboxyl group may have been diluted by an exchange reaction.

Probably in some organisms there will be an overlapping of heterotrophism and autotrophism, *i.e.*, the organism will be able not

only to perform syntheses such as the 3- and 1-carbon union but will be able to construct an entire chain from 1-carbon compounds. The case in question may be an example, for, as Barker *et al.* point out, Weiringa (1936) has shown that another species of *Clostridium* can synthesize acetic acid from CO_2 and hydrogen.

Finally, a few comments should be made relative to photosynthesis, for the close relationship between heterotrophic assimilation and the "dark reaction" of green plants has been clearly shown. It is becoming increasingly evident that chemosynthetic and photosynthetic CO_2 assimilation may be alike with exception of the process of reducing the fixed CO_2. Van Niel (1941) has made it highly probable that the different types of photosynthesis, some liberating oxygen, others not, are basically the same. Water acts as the hydrogen donator in each case, but in the one, oxygen is liberated from the resulting peroxide, while in the other a hydrogen donator such as H_2S reduces the peroxide without liberation of O_2. In photosynthesis, energy from the light is used to complete the requirements of the endothermic reaction

$$3 H_2O + CO_2 \rightarrow (HCOH) + 2 (OH)_2$$
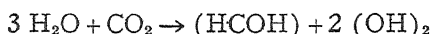
In chemosynthetic autotrophism this energy is supplied by chemical reactions, likewise, the resulting peroxide is reduced by hydrogen donators so there is no liberation of oxygen. Heterotrophic assimilation differs in that it requires organic hydrogen donators (*e.g., Athiorhodaceae* and perhaps *Cl. acidi-urici*). In the more advanced cases of heterotrophism the ability to perform the synthesis of an entire carbon chain from 1-carbon compounds has been lost.

The underlying mechanism of CO_2 assimilation may be the same in all forms of life. Certain heterotrophs apparently fix CO_2 by the following reactions:

$$CO_2 + HCH_2 \cdot CO \cdot COOH \rightarrow COOHCH_2 \cdot CO \cdot COOH$$
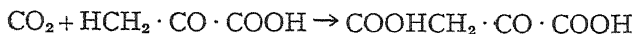
In a generalized form the reaction may be written

$$CO_2 + HCH_2 \cdot CO \cdot R \rightarrow COOH \cdot CH_2 \cdot CO \cdot R$$
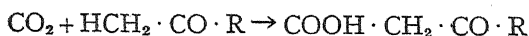
For the synthesis of a chain from CO_2 as in autotrophism the following reactions may occur:

$CO_2 + HCH_2 \cdot CO \cdot R \rightarrow COOH \cdot CH_2 \cdot CO \cdot R \xrightarrow{+4H, -H_2O}$

$H_2COH \cdot CH_2 \cdot CO \cdot R \xrightarrow{-H_2O} H_2C : CH \cdot CO \cdot R \xrightarrow{+H_2O}$

$HCH_2 \cdot CHOH \cdot CO \cdot R \rightarrow HCH_2 \cdot CO \cdot CHOH \cdot R$

$CO_2 + HCH_2 \cdot CO \cdot CHOHR \rightarrow$

$COOH \cdot CH_2 \cdot CO \cdot CHOH \cdot R$, etc.
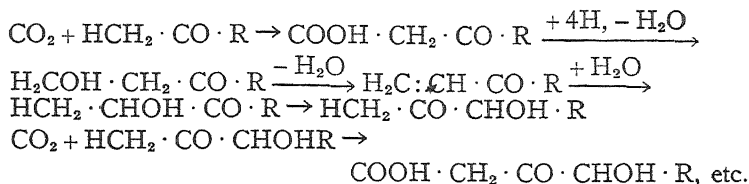
R equals the enzyme to which the chain is attached The carboxylation reaction, $CO_2 + HCH_2 \cdot CO \cdot \rightarrow COOH \cdot CH_2 \cdot CO \cdot$ would then be a general reaction of all assimilation.

Ruben, Kamen and Hassid (1940) and Ruben and Kamen (1940*b*) have presented a similar scheme, but the carboxylation is in an alcohol, $RCH_2OH + CO_2 \rightarrow RCHOH \cdot COOH$. The reader is referred to these experiments in which an attempt was made to isolate the intermediate products of photosynthesis using radioactive carbon, and to the reviews by Franck and Gaffron (1941) and van Niel (1941) for an understanding of present views of photosynthesis. Ruben *et al.* have found that the first detectable products formed in the light and dark by *Chlorella* have a molecular weight of about 1000. The compound has at least one alcohol group and one carboxyl group. The major part of all the $C^{11}O_2$ taken up in the dark is in the carboxyl group; the compound formed in the light, on the other hand, has a smaller portion in the carboxyl group. The carboxyl carbon was determined by heating the barium salt. The dark assimilation of CO_2 was found to be reversible and apparently independent of chlorophyll. This fact indicates that light is not essential for the primary step in photosynthesis, *i.e.*, the dark reaction. Ruben *et al.* were able to present some direct experimental evidence that the light reaction induces the reduction of the carboxyl group. They exposed *Chlorella* for twenty minutes to radioactive CO_2 in the dark, then replaced the $C^{11}O_2$ with ordinary CO_2 and illuminated it strongly for twenty minutes. The isolated barium salt was radioactive but no C^{11} could be detected in the carboxyl group. Apparently the $C^{11}O_2$ which was fixed in the carboxyl group in the dark reaction was reduced and then buried in the synthesized carbon chain by the subsequent photosynthesis occurring in the light.

It is apparent that during the short interval isotopes of carbon have been available, considerable progress has been made in an understanding of CO_2 assimilation. When this valuable tool be-

comes more universally available, and when the techniques for its use are more clearly understood and developed, it is certain that rapid strides will be made in our understanding of the chemistry of assimilation and biochemistry in general.

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THE CULTIVATION OF ALGAE

HAROLD C. BOLD
Barnard College, Columbia University

CONTENTS

INTRODUCTION

More than a decade has elapsed since the publication of Kufferath's (255) extensive paper entitled "La Culture des Algues." This compendium of valuable information reviews the history and progress of culture technic for algae during the 40 years preceding its publication. Due perhaps to the disadvantage of language, to its failure to correct important errors in formulae, to its omission of a number of important contributions which appeared during the period of review, and, finally, to the relatively limited circulation of the periodical in which it was published, Kufferath's paper is less valuable to American workers than it otherwise might be. In reviewing this same technical subject, the present writer has been motivated by the desire to overcome some of these disadvantages and to bring together in simple and convenient form, an outline of the methods which have played such an important rôle in the progress of morphology and physiology in the last decade. That this progress has been considerable is perhaps best indicated by stating that although Kufferath cites over 400 relevant papers published during the four decades preceding his review, the present writer finds it necessary to cite over 500, the majority of which have appeared during the past 12 years! The author is also convinced that the only possible excuse for reviewing such a technical subject is to make the information summarized of practical assistance in the laboratory, a purpose which has been dominant in the preparation of this review. It would of course be rash to claim credit for comprehensiveness, but no effort has been spared in attempting to cite all papers in any way involving the cultivation of algae, which have appeared since 1928 or which are not cited in the Kufferath paper.

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wishes to express his appreciation to Miss Dorothy Vormwald for her aid in compounding the culture media listed in Appendix I and for her zealous interest and help in preparing silica gels.

The literature on algae contains several contradictory concepts and usages of the term "pure culture." This appellation was first applied by bacteriologists following the methods of Koch, who were able to secure growth and multiplication of one type or species of bacterium in the absence of other micro-organisms. Such cultures are relatively easily obtainable for most saprophytic bacteria, and because they grow rapidly, if environment is suitable, they readily outstrip their fungus or protozoan competitors for "Lebensraum." A pure culture, then, originally signified some sort of container in which only one species of bacterium was present, and that, free from other living organisms. As far as the writer is aware, this interpretation is still generally accepted in bacteriological laboratories. Orskov (345), however, in 1922 defined a pure culture as one "consisting of individuals of which we know with certainty that all are descended from one single cell, and from one only." While theoretically in plating technics bacterial colonies which form the source of pure cultures are considered to have arisen from single cells, it is probable that they often arise from two or more recently divided cells. If these cells are of the same strain or species, pure cultures can still be obtained.

Unfortunately, in extending the term to include algal and fungus cultures, loose usage has resulted in confusion. Some of the earliest investigators, however, Beyerinck (33) and Miquel (315) among them, applied the term correctly, but not all their contemporaries were equally accurate. Tischutkin (460), for example, one of the first to employ agar in the cultivation of algae, states that he obtained 18 genera in "pure culture," but later in the paper he mentions the presence of bacteria in the same cultures. This may well have been an inspiration for similar usage which has continued throughout the literature. Tischutkin might more properly have referred to his cultures as "uni-algal" or "uni-specific," the former term being first suggested, however, a number of years later by Smith (442). This author clearly distinguishes between true pure cultures of algae, as those containing only one species

of alga free from all other organisms, and uni-algal cultures, as those containing but a single species of alga in the presence of other micro-organisms.   Smith here presents the generally accepted and classical view as to the categories of cultures and has been followed by a majority of foreign investigators (334, 363, 383, 406).

Pringsheim (383) in 1926 distinguished six types of culture, and these are to be interpreted as progressive, culminating in clone-cultures: *a*) Conservation- or Preservation-culture ("Erhaltungs-kultur") in which the individual organisms can be maintained in a condition similar to that in which they are found in nature; *b*) Crude- or Gross-culture ("Rohkultur") which one observes over a long period of time, noting the various organisms which appear; *c*) Accumulation- or Enrichment-culture ("Anhäufungskultur") with the medium so compounded as to favor development of one group; *d*) Species-pure culture ("Artreinkultur") containing a single species in such preponderance that it can not be confused with any other organisms present, a definition which is looser than Smith's (442) of "uni-algal culture," since it does not positively exclude other algae; Smith's term definitely does this and is, therefore, in the writer's opinion more accurate; *e*) Absolute pure culture ("absolute Reinkultur") in which individuals of only one species are present, free from all other organisms; *f*) Mono-cell or Clone cultures, ("Einzellkultur, Klonkultur") descended from single cells. The present writer would broaden the usage of this sixth term to include colonial organisms in cultures started from single colonies, provided these arise from single cells, as in the colonial Volvocales. Pringsheim points out that his sixth category may lie in either his fourth or fifth, as it is based on genetic criteria.   With the exception of the ambiguity in Pringsheim's definition of a "species-pure" culture, his classification is quite satisfactory.   Several modern workers, however, persist in using a different terminology (154, 431).

HISTORICAL ASPECTS

Famintzin (118) in 1871 seems to have been the first investigator to report the results of studies on the growth of algae in water cultures.   It was during the preceding decade that Knop (239–241) was publishing his series of papers dealing with the nutrition of flowering plants, and Famintzin experimented with the possibility of growing algae in one of Knop's (239) solutions.   The

following 20 years appear to have been relatively barren in the cultivation of algae, for it was not until 1890 that the Dutch bacteriologist, Beyerinck (33), became interested in the problem. At first, he followed the simplest and most obvious procedure, that of attempting to grow algae in water from their natural habitat, and in his classical paper describing the isolation of *Chlorella* and *Scenedesmus* in bacteria-free cultures, he used ditch water solidified with gelatine as the culture medium. The career of *Chlorella* as a botanical and physiological "guinea pig" was launched in this work. Beyerinck soon found it helpful to enrich water from the natural habitat with various inorganic and organic substances, and from this standpoint his work marked a return to the type of investigations of Famintzin, Knop and other students of the nutrition of flowering plants. In 1892 Noll (339) and Oltmanns (341) published papers discussing the cultivation of marine algae, but these dealt largely with maintaining the algae in good condition rather than with actually effecting growth and reproduction. Noll, however, advocated the addition of phosphorous and nitrogenous compounds to sea water, a practice which is followed by many present day investigators. Of equal importance to Beyerinck's studies of the Chlorophyceae are Miquel's (315) papers which appeared in 1892, describing methods for isolating diatoms in various types of cultures. Just as Beyerinck has received recognition for being the first to cultivate green algae in bacteria-free cultures, so Miquel is deserving of similar recognition with reference to diatoms. Kossowitch (246) claimed to have isolated *Cystococcus* in bacteria-free cultures in 1894 by means of the silica-gel technic. Richter (405, 406) continued with Miquel's methods and utilized pure cultures in physiological studies of diatoms. He further extended the work to other forms, and in 1911 presented an elaborate paper (407) summarizing all previous work on the nutrition of algae. Benecke (30) experimented on the mineral requirements of algae in 1898, but his cultures undoubtedly contained bacteria. At this early date, however, he advocated the use of water distilled from a platinum condenser. In 1900 Chodat and Grintzesco, and later each author separately (72, 73, 75, 151, 152), described their methods for obtaining pure cultures of algae. The investigations of Chodat and his students have been extremely fruitful in extending our knowledge of the physiology of the algae, and many of them bear on

the question of the constancy of species characters.   In 1903 Moore (328) summarized the methods employed up to that time for cultivating algae.   The paper by Chick (71) which apeared the same year is remarkable in many respects, and in the present writer's opinion has not received adequate recognition.   This investigator isolated *Chlorella pyrenoidosa* from bacteria-infested waters and grew it in pure cultures.   Furthermore, from time to time she tested the purity of the cultures by inoculation into bouillon, a medium favorable to bacterial growth.   She also noted that glucose greatly accelerated development and that the alga seemed to prefer ammonia nitrogen to oxidized forms.   Finally, she tested the culture medium for the presence of nitrogen compounds and concluded that if such substances are present, they originate from the decomposition of dead cells rather than as secretions of living ones. In 1912 Pringsheim (374) published the first of a series of papers reporting the results of his researches on the cultivation of algae; these have continued uninterruptedly to the present day.

The publications of Pringsheim and his associates deal with methods of cultivation as well as with physiological and morphological features.   Among them are several lists offering bacteria-free cultures at a nominal cost for the use of investigators throughout the world.   Many studies dealing with the physiology of nutrition and growth, of photosynthesis, and the effects of growth substances are based on pure cultures obtained from the Prague laboratory.   Until 1938 this laboratory was a center for the distribution of a large number of algae maintained in pure or uni-algal culture.   The present world situation will undoubtedly affect the shipment of these cultures.   Schramm (428) in 1914 published an excellent summary of culture methods for algae in general and of methods for certain special organisms.   Nakano (334) presented an important table summarizing the extent of pure culture work on algae up to 1917, and a similar summary up to 1929 may be found in the paper by Mainx (299).

One of the most important factors in the development of the technic of cultivating algae was the introduction of the use of soil extracts.   Pringsheim (374) was one of the first to report this, although Karsten used it earlier in the cultivation of diatoms.   The addition of small quantities of soil extracts to culture media has made possible the cultivation of many genera which had hitherto

resisted all attempts to cultivate them in the laboratory. Moewus' (323) recent discovery that soil extracts prepared without heat, greatly increase the percentage of zygote germination, is an important advance in the soil extract technic. Finally, Meier (306) has published a brief but interesting historical treatment dealing with the cultivation of algae.

<div align="center">TECHNIC</div>

In general, the methods of cultivating algae are those of the bacteriologist, and differences are due to certain idiosyncrasies in the rate and habit of growth, in size, in thermal tolerance, or in the metabolism of the algae. Proficiency in and familiarity with elementary principles of bacteriological technic are practically indispensable pre-requisites to success in cultivating algae; abundant patience is either pre-requisite or must be developed. There are numerous works dealing with culture methods for algae, many of them portions of more general volumes on the technics of microbiology (43, 60, 72, 73, 75, 128, 158, 233, 244, 248, 255, 259, 261, 299, 306, 320, 328, 374, 381, 383, 407, 423, 426, 428, 439, 486, 489); they contain valuable information, both generalized and dealing with special groups.

Glassware. All investigators emphasize the importance of using only chemically clean glassware; this is readily obtained by means of the various cleaning solutions available in the laboratory, use of which should be followed by thorough rinsing in tap and distilled water. The glassware should then be dried and stored free from dust. The culture vessels themselves should preferably be of Pyrex or some other high grade glass and should not be used too long. Hartmann (181) reports that glassware in use more than two and a half years was harmful to cultures of *Eudorina elegans*. Pringsheim (382, 388) recommends Jena glass, and Czurda (88) found it necessary to use a special alkali-free glass in his work with *Spirogyra*. Microscopic algae in either liquid or solid media, may be grown in test- or media tubes, Petri dishes, small Esmarch dishes, Syracuse watch glasses, or in flat, screw-top medicine bottles. For careful physiological work the highest quality glass should be employed. Ondraček (342) has performed careful experiments using various kinds of glassware in the cultivation of algae.

Inorganic culture solutions. Obviously, the simplest and most readily available culture medium that suggests itself is the natural liquid in which a given organism is growing, provided it is an aquatic form (33). Many earlier investigators employed tap water as a substitute (234), with more or less success. The widespread use of tap water in the infancy of phycological technic is probably correlated with the prevalence of wells and springs as sources of supply, in close proximity to the laboratory, and the absence of chlorination treatments and brass plumbing. The development of modern plumbing introduced complications in the form of metal salts. Chlorine can be removed readily by exposing the water for some time after it is drawn and before the algae are submerged in it, or by boiling or heating to drive off the gas (200). Metals in water are very destructive to algae, and these lethal agents will become increasingly prevalent with the extensive installation of brass pipes in modern buildings. Brandwein (49) has suggested a medium containing tap water for the culture of *Spirogyra.* The writer was consistently unsuccessful in using it until he treated the tap water with animal charcoal and subsequently filtered it. It is clear in the present case that the toxic substances are introduced from the plumbing system in the laboratory, since water taken near the city main does not inhibit the growth of *Spirogyra,* provided the water is dechlorinated. In connection with the problem of toxicity of water, there are several instructive discussions in the literature. Livingston (277) long ago suggested improving distilled water with thoroughly washed carbon black. Allen and Nelson (7) shook animal charcoal and sea water to prepare the latter for growing diatoms. The literature of the chytrids indicates that some variety of charcoal water is often used as a culture medium. Hoyt (200) performed rather extensive experiments on the effect of various treatments of water on the growth of *Spirogyra.* He reports that heating tap water for 15 minutes in the autoclave at 140° C. destroyed all toxic substances; apparently, the water in question contained only toxic gases or at least not metal impurities. He also recommends introduction of charcoal into the retort in distilling water, a practice followed recently by the writer. Steinberg (446) introduced 15 grams per liter of calcium carbonate into his culture solution, decanting the liquid after sterilization, in order to remove heavy

metals.   Subsequent to such treatment he added known amounts of iron, manganese and other elements.

Toxicity effects are not limited to tap water alone but occur often in ordinary distilled water when it is prepared from certain types of metal condensers.   Modern workers are almost unanimous in their preference in critical work of water re-distilled through Pyrex or quartz condensers.   Distilled water can not be used generally as a culture medium because of the absence of nutritive elements, but Gojdics (148) reports that *Euglena deses* grew and multiplied for six weeks in distilled water alone.   It seems probable that small quantities of nutrients were transferred into the distilled water along with the original inoculum.

The numerous and diverse formulae for inorganic culture solutions appearing in phycological literature reflect the conclusions of various experimenters as to the nutritional requirements of algae. With the possible exception of calcium, however, algae are probably quite similar to higher green plants in their requirements, and, therefore, familiarity with such general texts as that of Miller (313) is an asset.   The many algal culture solutions referred to, differ only in minor respects, as in concentration, source of the nitrogen, potassium, calcium, iron, *etc.,* variations which have been developed in cultivating specific organisms.   The writer does not feel that it would be valuable to review all such variations in the present paper, since they are included to a large extent in the summaries of Kufferath (255) and Mainx (299).   Appendix II contains citations to papers dealing with the specific requirements of individual organisms.   There are features of nutrition of the algae, which, however, merit some discussion.

Not the least of these is the great confusion which exists in the literature due to the practice of designating solutions by the names of their supposed original users or compounders, without verifying the composition and source of the formula or the date of its first publication.   Even reviewers (255, 299) are guilty of this, and the present writer has hitherto erred in a similar manner (43).   Tottingham (462), in his exhaustive study of Knop's Solution, points out that there are a number of different verisons of this medium, and he has gone back to the original source as the foundation of his work.   The history of the so-called "Benecke's Solution" is a case in point, and the writer feels it essential to clarify a most confusing situation.

"Benecke's Solution" has been widely used and seems to be outstanding as a general culture medium for many algae. There are, however, at least two very different formulae designated in the literature as "Benecke's Solution," and, as will become clear below, Benecke is responsible for only one of them—the one less widely used, possibly because it was compounded originally for growing *Elodea!* Kostka (248), in his compendium of culture methods for micro-organisms, lists a formula for "Benecke's Solution" (p. 133) and one for "Benecke's Agar" (p. 140) which differ not only in the obvious presence of agar in the latter, but also in the salt ingredients, qualitatively and quantitatively. Furthermore, the source of the first is given as page 87 of Benecke's paper (30), on which page this solution is mentioned in connection with experiments on *Elodea.* No citation is given by Kostka for the formula for "Benecke's Agar," nor is any explanation offered for the discrepancy in the formulae. It is this formula, however, without the agar, which is widely used as "Benecke's Solution." Mainx (299) and Kufferath (255) both list "Benecke's Solution" as containing the liquid and salt ingredients of Kostka's "Benecke's Agar" without citing an original source. Kufferath (255) offers two different formulae which differ only in concentration (p. 46). Hartmann (181) has contributed to the confusion by an error in the spelling of Benecke's name (which the writer repeated, 43), by failing to cite a source for the solution and, incidentally, by an error in the iron constituent of Knop's Solution. In 1928, however, he corrected the spelling (233) but stilled failed to cite a source. It is all the more regrettable that so much confusion exists concerning the widely used variety of "Benecke's Solution" (see Appendix I) especially since it was Beyerinck (34) who devised it originally as a medium for *Pleurococcus!* The solution should, therefore, properly be designated "Beyerinck's Solution, 1898," and a citation of its source should always be given. Several investigators (158, 255) have used this solution under the correct title (Beyerinck's) without realizing that the "Benecke's Solution" of some other authors was identical with it. In the writer's opinion, a more accurate system of citing culture solutions is clearly necessary. One further point seems noteworthy: the majority of papers fail to specify whether or not anhydrous salts have been utilized. If the water of hydration is not given, it is generally assumed by the

writer that hydrated salts were employed. Tottingham (462) urges the use of anhydrous salts, especially calcium nitrate, in the interests of accuracy; it would certainly make a material difference in the concentration of nitrate ions in Knop's Solution, should one follow his suggestion. A more detailed discussion of the last-named and other solutions is included in Appendix I.

Nutritional Requirements. Although, as noted above, a knowledge of the salt requirements of the organism is pre-requisite to compounding the proper culture medium, it is beyond the scope of the present review to deal extensively with this subject, the more so because excellent summaries are already available (299, 313, 324, 325, 407). There is considerable evidence that calcium is unnecessary for certain algae (324, 382, 463). The writer has obtained thriving cultures of *Chlorella* which contained no calcium other than the minute amounts which may have been introduced as impurities in the chemicals. Potassium seems to be necessary and irreplaceable for green algae (112, 324, 382).

Phosphorus, magnesium and sulphur ions are regularly included in all culture media, as well as nitrate or ammonium ions. There is an increasing amount of carefully controlled work which indicates that certain Myxophyceae have the capacity to combine atmospheric nitrogen (8, 9, 81, 96, 106, 326, 484, 498), and that they can grow in culture media lacking a source of combined nitrogen. This ability seems limited to the Myxophyceae, attempts to demonstrate a similar activity in the Chlorophyceae having led to negative conclusions in every case (61, 246, 253, 429). A few investigators report that nitrite may be suitable as a source of nitrogen (11, 15, 18). Artari (15), however, states that potassium nitrite is more suitable if it is allowed to stand for some time before use; this perhaps indicates an oxidation of the nitrite to nitrate. Earlier papers often debated the relative merits of ammonium and nitrate nitrogen in solutions. The widespread use of Knop's Solution, containing only nitrate nitrogen, and Beyerinck's Solution, containing only ammonium nitrogen, for the same organisms, as indicated by a survey of the literature, furnishes important evidence that either type of nitrogen is suitable, other factors, pH for example, being favorable. Pratt and Fong (372) have recently demonstrated that *Chlorella* absorbs ammonium nitrogen more rapidly than nitrate nitrogen when both are present in the solution;

but growth is best when all of the nitrogen is in the form of nitrate. The medium becomes more acid in solutions containing a high proportion of ammonium and less acid when nitrate alone is present, regardless of the initial pH. Many papers contain data on the availability of both inorganic and organic nitrogen sources for algae (5, 14, 15, 25, 28, 46, 71, 84, 88, 110, 166, 281, 283, 288, 299, 372, 440, 493).

Although many culture solutions do not specifically require the addition of iron it is undoubtedly introduced as an impurity in other salts. Uspenski and others (453, 469–473) have emphasized the important rôle of this element in the culture solution in determining form, and incidentally in affecting the distribution of algae in nature. Roberg (411) found that autotrophic Chlorophyceae could not grow in the absence of iron. Hopkins and Wann (198, 199) have investigated the availability of iron in relation to pH and suggest the use of sodium citrate to effect the availability of iron in alkaline solutions. Several workers now add iron in the form of iron citrate because of the buffer action of the latter, or obtain the same result by adding a little potassium citrate to a solution containing an inorganic salt of iron.

The so-called "micro-metabolic" elements are undoubtedly important in the nutrition of algae, and should probably be included in all culture solutions, in spite of the possibility of their introduction as impurities in other salts. Hopkins (197) has proven the indispensability of manganese for green plants. Trelease and Trelease (464) have devised a solution containing the "micro-metabolic" elements necessary for higher plants, and have modified it for cultivating Chlorella (82, 464, Appendix I). This micro-element solution may be added in a concentration of 10 cc. to the liter to avoid deficiencies in ordinary culture solutions.

Media containing organic ingredients. Immediately after isolating his Chlorella and Scenedesmus, Beyerinck (33) experimented with various carbohydrate and organic nitrogen compounds as supplementary sources of nutrition, and concluded that growth in media supplied with certain sugars was far superior to that in strictly inorganic media. He thus laid the foundation for a long series of papers, too numerous to cite, dealing with attempts to test various organic compounds as nutrients for algae. The use of organic ingredients in the culture medium is unsatisfactory, if not

impossible, in cultures contaminated with bacteria. It is obvious that no conclusions as to the relations of algae to organic materials are valid unless they are based on work with bacteria-free cultures. Furthermore, Skinner and Gardner (440) point out that such experiments should be performed in silica gel or in liquid media because of the impossibility of ridding agar of organic nitrogen.

The rôle of organic compounds in the metabolism of algae is of course merely a portion of the larger problem of the nutrition of the Protista in general. Hall and his associates (167–169, 281) have reported on a series of comprehensive experiments dealing with the nutrition of the "plant-like flagellates," and he has recently (167) reviewed the classification and terminology of various types of nutrition. Lwoff (289) has reported on similar experiments. The higher green plants and algae are usually regarded as having an autotrophic mode of nutrition, as contrasted with the heterotrophic nutrition of fungi. The habitat of a green plant, whether in soil or in water, normally contains abundant and diverse organic substances, and it is very probable that green organisms absorb and utilize such materials. On *a priori* grounds, therefore, one would expect chlorophylliferous plants to possess the capacity for facultative saprophytic nutrition. The carefully controlled experiments of Hall and his associates bear out this expectation and indicate that exclusively autotrophic nutrition among the "plant-like flagellates" is rare. In connection with their nutritional studies, Hall and Schoenborn (169) have discovered that even in pure cultures of *Euglena gracilis, E. minor* and *E. viridis,* individuals apparently differ in their capacity for "photoautotrophic" nutrition, and they have been able to develop a "photoautotrophic" race by selection. These experiments are significant in any consideration of the phylogenetic position of such organisms.

The relation of algae to carbohydrates supplied in the culture medium is summarized in the excellent table by Mainx (299). The entire literature on this subject is too extensive to be discussed at length in the present paper, but the following observations are worthy of note. Artari (15), using pure cultures of *Stichococcus, Chlorella* and lichen gonidia, was among the first to carry out extensive experiments on the availability of various carbon compounds to algae in light and darkness. He found that glycerine,

erythrite and mannite were moderately useful; glucose, galactose, fructose, saccharose, maltose and inulin more so; rhamnose and mannose were practically of no value. Bristol-Roach (57, 59), in a careful study of the relation between some soil algae and soluble carbon compounds, concluded that *Scenedesmus costulatus* var. *chlorelloides* is capable of growing in the dark at a rate about 40% as fast as in the light, provided it is supplied with glucose, and she interpreted this as an indication that the organisms can grow in deeper layers of soil, provided suitable carbon compounds are available. She extended these observations the following year (58). Czurda (85) reports that pure cultures of *Mesotaenium* are able to utilize a number of carbohydrates in light or darkness, while *Cosmarium, Zygnema* and *Spirogyra varians* can not do so. Luksch (288) also has carried on extensive experiments on the relation of chlamydomonads to carbohydrates. Loefer (282), in the report of his study of the influence of carbohydrates on the growth of *Chlorogonium,* has presented a table summarizing the effects of carbohydrates on protozoa in general. Loefer and Hall (286) report a stimulation of the growth of *Euglena gracilis* by alcohol in concentration of 0.25 to 1.0%.

Chick (71), as early as 1903, performed a number of experiments on the relation of *Chlorella* to various organic nitrogen sources. Mainx (299) has summarized in tabular form the relation of algae to various sources of organic nitrogen. Skinner and Gardner (440) have demonstrated that casein, albumin and glucose may serve as the sole source of energy for pure cultures of green algae in darkness. Dusi (110) has reported that various *Euglena* species have the ability to use amino acids, and Loefer (283) reached similar conclusions with respect to *Chlorogonium.* The widespread use of dilute solutions of meat extract as a medium for maintaining pure cultures of algae indicates the value of organic nitrogen sources.

A number of other more complex organic ingredients are recommended for inclusion in certain culture media. One of the most generally used is soil extract or decoction, the preparation of which is discussed more fully in Appendix I. Similarly, decoctions of hay and grass, manure, alfalfa, rice, kelps, *etc.,* are reported as stimulating the growth of certain algae. Reference to the papers cited in Appendix II will afford more specific information.

Pringsheim (374), who is usually credited with having been the first to use soil extracts, has recently (388) discussed them and the reasons for their marked stimulation of the growth of most algae. He points out that many algae which can not be successfully cultivated in other inorganic and organic solutions, will grow luxuriantly if a small amount of soil extract be added. On the basis of experiments employing *Polytoma, Chlorogonium, Polytomella* and *Chilomonas* as test organisms, he has concluded that the active principle is an acid- and alkaline-stable organic substance, insoluble in alcohol and ether, adsorbed by charcoal, and destroyed by hydrogen peroxide. Various humus derivatives, like Waksman and Iyer's (488) iron-ligno-protein compound, exerted a stimulating effect on the cultures.

Solid Media. Practically all culture solutions mentioned above and in Appendix I may be solidified to any desired degree by using one of the agents discussed below. Probably the earliest recorded example of this practice in phycology is Beyerinck's (33) use of gelatine to solidify ditch water media in 1890. Gelatine may still be employed, but it has largely been supplanted by agar for several reasons. In the first place, gelatine media must be stored at relatively lower temperatures than agar of the same concentration in order for them to remain solid and thus prevent discrete algal colonies from becoming confluent. Secondly, algal cultures containing bacteria are usually unsuccessful on gelatine media because so many of the contaminating organisms rapidly liquefy the gelatine. Finally, at least a few algae themselves seem to liquefy this substance (33, 112, 215, 334, 455).

Tischutkin (460) in 1897 and Beyerinck (34) in 1898 were among the first to use media solidified with agar for cultivating algae,[1] and this expedient seems to be the simplest and most useful at present. Although the literature abounds in evidence to the contrary (79, 121, 122, 175, 259, 291, 326, 396, 408, 440), it seems to be overlooked in many quarters that agar is not a completely inert substance which, when added to a given culture medium, simply changes its consistency without affecting its chemical composition, osmotic properties or acidity. If agar were inert it would be very simple to obtain bacteria-free cultures of algae by simply adding agar to a medium containing only inorganic salts. Theoretically,

[1] Hitchens and Leikind (190) have recently reviewed the history of the use of agar in bacteriology.

if mixed cultures containing bacteria, fungi and algae were inoculated into such a medium, the algae would find themselves in completely congenial surroundings from the standpoint of nutrition, while the bacteria (provided they were not of the type which digest agar nor chemosynthetic) and fungi would be destined to early destruction by inanition. One should thus be able to take advantage of the difference between autotrophic and heterotrophic nutrition in isolating algae on solid media. The writer has attempted during the past ten years to take advantage of this difference in freeing *Protosiphon* from unwelcome fungi and bacteria, but has always obtained relatively luxuriant growth of the latter groups, with simultaneous inhibition of the *Protosiphon*. This is the common experience of everyone who has attempted to culture algae. At least two explanations immediately suggest themselves, and undoubtedly both together operate in such impure agar cultures.

In the first place, as noted above, there is a great deal of evidence that agar is not at all an inert substance. As early as 1888 Macé recommended a method for freeing agar of impurities. Pringsheim and Pringsheim (396) in 1910 demonstrated that agar could be used as a source of energy for nitrogen-fixing organisms. Küster (259) noted that agar alone possessed sufficient nutriment to support the growth of some organisms, and suggested methods of purification. Fellers (121, 122) in 1916 published two very important and currently neglected contributions in this connection. The first, a chemical study, contains a summary of early chemical analyses of agar as well as his own, and these indicate the universal occurrence of nitrogen compounds, carbohydrates and other substances in agar samples. Furthermore, the agar itself affects the acidity of a culture medium. The second paper (122) is an excellent and careful study of agar as it is used in bacteriological (and hence phycological) technic; it demonstrates beyond question that "impurities" revealed by chemical analysis are available for the nutrition of bacteria and fungi. Harder (175) remarks that even washed agar contains traces of organic materials available to microorganisms. Similar conclusions are reported by others (79, 259, 326, 440). Quite recently Robbins (408) has demonstrated a stimulative effect of commercial Bacto-Difco agar on the growth of *Phycomyces,* in comparison with growth in similar media solidified with purified agar. He is inclined to ascribe the stimulation to the

action of growth substances present in agar. That agar is not inert should undoubtedly be given greater consideration in work with *Avena* coleoptile and its extracts. The facts here summarized offer one possible explanation of the failure of micro-organisms with heterotrophic nutrition to be eliminated from agar media containing only inorganic ingredients.

A second explanation which may account for the growth of bacteria and fungi in inorganic culture solutions lacking agar, is that the algae supply certain organic substances useful to heterotrophic organisms. These may be waste materials thrown off by the living algal cells or compounds secreted during anabolism (photosynthesis, *etc.*), or materials made available by the death of some of the algal cells. It is the opinion of the writer that, at least on theoretical grounds, it is not unreasonable to assume that some materials diffuse from the algal cells into the surrounding medium. This is certainly to be assumed in lichens where haustorial connections between fungus and alga have not been demonstrated. He believes that hexose sugars might be among such diffusible substances and that they would stimulate growth of bacteria and fungi, even if only minute amounts entered the culture medium. Pütter (398, 399) seems to have been the first to suggest that such diffusion from submerged plants is an important factor in the nutrition of aquatic animals. This hypothesis has been denied (71, 149, 252, 303), but the careful work of certain investigators (46, 96, 131, 370, 371) indicates, nonetheless, that it may be justified. Roberg (410), for example, concludes that pure cultures of algae do secrete organic matter into purely inorganic culture solutions, although he was unable to obtain a positive test for sugars. Braarud and Føyn (46) report that about 30% of oxidizable photosynthate diffuses from the living cells of algae into the culture medium. De (96), in a recent account of nitrogen fixation by Myxophyceae, concludes that some of the fixed nitrogen appears free in the culture medium! In this connection, the papers of Pratt (371) and Pratt and Fong (372) are significant. These investigators report the accumulation of inhibiting substances in cultures of *Chlorella;* the culture medium when freed of cells by filtration and reinoculated acts in an inhibitory manner. Since such cultures, although apparently not bacteria-free, are maintained under optimum conditions of salt nutrition, carbon

dioxide availability, light and temperature, it may be inferred that few cells are dying, so that the inhibiting substances must represent excretions from living cells. On the other hand, others (4, 71, 252, 303) conclude that organic matter appearing in inorganic culture solutions containing algae, represents products liberated by decay of dead cells. Gaarder and Gran (131) state that there may be some truth to Pütter's conclusions, but later, Gran (149) states that soluble organic matter plays only a minor rôle in the nutrition of marine animals. This whole problem is of course involved in the question of the nutritional relations between the symbionts in lichens and in "green animals," in nodule associations and in space parasitisms in general. On the basis of the evidence summarized in this paragraph, it seems to the writer that even if agar were completely inert, it would still be impossible, on the basis of differential nutrition, to eradicate heterotrophic fungi and bacteria from algal cultures in inorganic media; the heterotrophic organisms would probably obtain organic nutrient materials from the secretions of algal cells or the products of their decay. Such considerations discourage the use of silica gels for the same purpose, as will be pointed out later. Ronse's (414) recent report that *Cosmarium* grows better in the presence of certain bacteria than in pure culture, is significant in connection with the above discussion.

In spite of the impossibility of obtaining absolutely inert agar by available methods of purification, there are several advantages which result from even partial purification. Washed agar remains more transparent after solidifying than does untreated commercial agar. This property makes it more valuable in the microscopic examination of plates and in transferring colonies. Furthermore, as Robbins (408) has demonstrated, growth of fungi is less luxuriant on purified agar. Once one has obtained pure cultures of algae, there is no longer any advantage in purifying agar, but it is, on the contrary, distinctly disadvantageous, since the very substances removed in the purification undoubtedly stimulate the growth of algae.

Perhaps the simplest treatment is that suggested long ago by Beyerinck (34) and Küster (259) and recently by Cohen (79). This consists of washing the agar, previously weighed and confined in a cheesecloth bag, for one to several days in running water, then squeezing out the excess tap water, and soaking for several

days or hours in a number of changes of distilled water. The nutrient medium may then be added to the moist agar, provided a correction is made for water absorbed by the agar, or the latter may be dried in the air or oven, cut up into shreds and dissolved in the full amount of nutrient solution. Various investigators (17, 157, 227) recommend soaking agar in dilute (1 to 2%) hydrochloric acid for several to 24 hours, rinsing thoroughly in water, and proceeding subsequently as noted above. Some follow the hydrochloric acid and washing with a period of soaking in weak ammonia (1%) solution, and then proceed as above. Too great a concentration of hydrochloric acid reduces the ability of the agar to gel. Macé (291) emphasized the presence of impurities in agar and seems to have been the first to recommend improving it by treatment with hydrochloric acid. Keding (227) modified this method slightly by substituting caustic potash solution for ammonia in preparing agar for studies of nitrogen bacteria.

Fellers (122) outlines a method which reduces the original nitrogen content of an agar sample by from 60% to 93%. This consists of treatment with ether, alcohol to remove the ether, soaking in acetic acid, thorough rinsing in distilled water, preparation of the agar as a 5% solution, subsequent autoclaving, filtration through cotton, precipitation of the hot agar in 95% alcohol or acetone, and final drying of the agar. Robbins (408) employed another method involving treatment with pyridine. He kindly gave the writer some agar purified by this method, and from observations of the growth of impure cultures of *Protosiphon* on this and media compounded with ordinary agar, it became very evident that purification of the agar effects a marked reduction in growth of fungi and bacteria. Hoffman and Gortner (191) have developed a method of purification by electro-dialysis, which, however, seems to reduce the gelling property of the agar.

The concentration of agar usually recommended varies from about 0.7% to 3.0% by weight and volume, depending on the final consistency desired. Moweus (317) found that weaker agar gels produced the largest *Protosiphon* cells. The writer finds a concentration of 1.0% satisfactory for general use. If the agar is purified by washing, it seems a wise precaution to begin with a 1.5% solution. The necessary concentration of agar is also correlated with the pH of the medium, acid conditions reducing gelation capacity.

As noted above, one important disadvantage in using agar as a medium for plating out algae in attempting to obtain pure cultures, is the presence of organic impurities in the agar which support a fairly luxuriant growth of fungi and bacteria. To avoid this, a number of investigators (196, 350, 376, 378, *etc.*) have turned to silica jellies as the bases for solid media. Direction for preparation of the gel are sometimes included in such phycological studies, and, in addition, a number of papers has been published which deal especially with this topic (27, 174, 241, 487). In the writer's experience, it has been fairly simple to make sufficiently rigid gels, using hydrochloric acid and almost any brand of sodium or potassium silicate, either in crystalline form or as commercial solutions, provided one employs sufficiently concentrated solutions, such as approximately normal acid and silicate. However, since such gels contain concentration of chlorides which inhibit growth of most micro-organisms, dialysis is necessary. After this process has proceeded sufficiently, the gels are often soft and easily broken and tend to liquefy if autoclaved or even if sterilized in flowing steam.

Batchelor (27) has recently discussed the factors which influence the preparation of silica gels, and his methods, in the writer's opinion, are vastly superior to any other yet described for use in cultivating algae. The chief advantages lie in the avoidance of dialysis, the direct incorporation of the nutrient solution in the gel, and in the ability of the finished product to successfully withstand sterilization in the autoclave. In Batchelor's experience and the writer's the factor which most frequently determines success or failure, is the type and brand of silica gel employed. For general purposes, potassium silicate seems to be superior to that of sodium (27); the writer has successfully prepared gels with "Grasselli potassium silicate solution 30°" while three other brands failed to give satisfactory results when treated in identical fashion. Dr. Batchelor [in correspondence] attributes failures with certain brands of potassium silicate to the fact that they have relatively high alkaline and low silica content.

The author has obtained the most satisfactory results by using Batchelor's method and Beyerinck's Solution, 1898 (see Appendix I), since the pH of the latter is approximately 7.2; Detmer's and Knop's Solutions are more acid and therefore least satisfactory for use with diluted gels. The potassium silicate solution is diluted

with distilled water until equal volumes of silicate and normal hydrochloric acid give a neutral reaction with bromthymol blue (pH 7.0). For the current supply of silicate it was found necessary to dilute 5.2 parts of the commercial product with 4.8 parts of distilled water. Ten cc. of normal hydrochloric acid are placed in a beaker or flask, the desired amount of Beyerinck's Solution added, and the whole mixed with 10 cc. of the diluted potassium silicate. The beaker is rotated to effect thorough mixing, and approximately 30 cc. portions are then poured into Petri dishes. These may be sterilized in the autoclave or Arnold steamer. The latter is more satisfactory, since less ammonia seems to be liberated and bubbles are not so large and numerous as to crack the gel. The finished plates may then be inoculated by gently streaking with a glass rod dipped in the inoculum or with a wire loop.

The greater the amount of nutrient solution employed, the greater will be the dilution of the undesirable chlorides. Thus, when 60 cc. are used, the chloride content is approximately 0.9%, while when 100 cc. are used, it falls to 0.6%. Concentrations such as these do not seem to inhibit the growth of *Chlorella*. While gelation occurs more rapidly at higher temperatures, it is less satisfactory to employ them with Beyerinck's Solution, since the ammonia is driven off by prolonged heating and gelation fails to occur, due to increased acidity of the nutrient solution. The solutions were therefore mixed at room temperature so that the plates took longer to "set." Obviously, the gels are firmer when less nutrient solution is employed; 60 to 90 cc. of solution added to 10 cc. each of acid and diluted silicate produces a satisfactory gel.

The writer has also successfully used silica gel in plating out by simply mixing the desired concentration of inoculum with the mixture just before the plates are poured. In this procedure sterile Petri dishes, flasks and pipettes were employed, although no attempt was made to sterilize the acid, silicate or Beyerinck's Solution. It is very important to free the algal cells from as much as possible of the original medium, since slight traces of organic matter carried into the gel with the inoculum are sufficient to support growth of bacteria and fungi until the algal colonies are large enough to afford a source of supply. This may be accomplished by centrifuging the algal cells and rinsing through a number of changes of sterile, inorganic culture medium or distilled water. In spite of

such precautions, sufficient nutrients are present to support a
meager growth of fungi and bacteria, so that except in special cases
the writer has concluded that plating out in agar is a more gener-
ally useful technic.   Finally, it should be stated that it is possible
to introduce most of the required nutrient salts into the gel by
using a mixture of hydrochloric, sulphuric and phosphoric acids,
while the other necessary ions may be added in the dilution fluid.

Various other solid substrata have been utilized by investigators
for specific purposes. Czurda (85) found it necessary to grow cer-
tain desmids and *Zygnema* on filter paper strips partly submerged
in tubes of liquid media.   Mainx (295) used a similar method for
Eremosphaera, and it is also suggested for desmids by Krieger
(251).   Iggena (205) cultured soil algae on glass wool substrata.
Gypsum or plaster of Paris blocks may be fashioned in various
shapes and sizes and immersed in nutrient solutions.   Schindler
(424), Glade (147) and Fechner (119) report successful cultiva-
tion of Myxophyceae in this way.   Miller (314) and Knebel (236)
used sterilized sand and clay moistened with nutrient solution for
Botrydium and *Prasinocladus,* respectively.   Livingston (278)
has described a porous clay cup for growing algae and moss proto-
nemata over long periods.

Illumination of cultures. It is almost universally conceded that
direct sunlight is harmful to algal cultures.   They are, accordingly,
shaded from such rays, or more often placed in a "north light"
where they are never in direct sunlight.   Probably, sunlight *per se*
is not harmful, since algae in nature often grow exposed to sunlight
of great intensity.   However, in small culture vessels the concen-
tration of heat in direct sunlight is apt to exceed the tolerance of
most algae.   Pocock (368) states that direct sunlight is necessary
for the germination of the oospores of *Volvox Rousseleti.*   A num-
ber of modern investigators still achieve successful cultures by
using north light (12, 90, 105, 222, 331).   A majority, however,
have resorted to artificial light of some kind.   Some go so far as to
maintain their cultures in basement dark rooms, using artificial
light exclusively, and incidentally taking advantage of the low
temperatures under such conditions (156, 242).   Various devices
for artificial light sources have been suggested, the main difficulty
being the maintenance of relatively low temperatures.   Warburg
(491) describes and figures an artificial light source much like that

described later by Hartmann (181) and slightly modified by the writer (43). It consists of a 500-watt tungsten-filament lamp suspended in a Pyrex beaker which is in turn suspended in a larger glass vessel containing circulating cold water. Many cultures can be maintained with such a light source, especially if glass shelves are employed. Essentially such a device has been used for a number of years in experiments with *Chlorella* (82) at Columbia University. Optimum light intensity is approximately 10,000 meter-candles measured with a photo-electric-cell light meter, but growth is excellent between 5,000 and 20,000 meter candles, according to Professor Trelease (unpublished data). Pringsheim (383) recommends this type of illumination device as "Hartmann's," but on grounds of priority it should be designated as "Warburg's."

Juller (221) suspended a 500-watt bulb above his cultures and separated it from the latter by a glass plate smeared with glycerine. Ketchum and Redfield (229) conducted their work with *Nitzschia* cultures with cool illumination from neon lights. The new fluorescent lights seem to supply an excellent source of cool and intense illumination (336a).

Several investigators have studied the effect of light on the growth rate of algae (205, 364, 445). Philip (364) reports that polarized light from the moon stimulates reproduction in *Nitzschia paradoxa* by promoting hydrolysis of starch with diastase, thus supplying energy for nuclear division. Iggena (205) has recently studied the effect of day length on various green and blue-green algae. He found that different day lengths apparently had no effect on 18 strains of Myxophyceae from habitats with markedly different day lengths; all of them grew more rapidly with increased exposure to light. As noted elsewhere, many algae are able to grow for indefinitely long periods in complete darkness, provided they are supplied with glucose or other carbohydrates or proteins in substitution for the missing photosynthate (94, 440).

Temperature. Investigators differ markedly in their reports of optimum temperatures for cultivating various algae. The writer has already pointed out that the chief danger of sunlight probably lies in the accumulation of heat in small culture vessels. Among the highest temperatures recommended for growing algae is that reported in the papers of Loefer (282–284); in his experiments on the nutrition of *Chlorogonium* he maintained the cultures in a water

bath at a constant temperature of 28° C. At the other extreme perhaps are the methods of Hygen (204), Herbst and Johnstone (187), Gross (156) and Kohler-Wieder (242). Hygen grew his bacteria-free cultures of *Nematocystus* at temperatures of from 2° to 12° C. Herbst and Johnstone (187) recommended temperatures of 8° to 12° C. as optimum for the development of the gametophytes of *Pelagophycus,* while Gross (156) obtained satisfactory cultures of *Ulothrix zonata* only when the temperature remained below 10° C. Kohler-Wieder found that 12° C. was best for culturing *Glenodinium,* while Barker (26) grew cultures of other dinoflagellates at temperatures of from 18° to 25° C. In most papers where temperature is not especially emphasized it may be assumed that room temperature, probably 18° to 25° C., depending on the season, has proven satisfactory. In general, the temperature should be maintained at a level comparable to that of the natural habitat of the organism. Craig and Trelease (82) stored their cultures at 12° C. preceding their experiments on photosynthesis; they later found that thriving cultures may be maintained for weeks at 5° C. with very little change in cell number.

Aeration of cultures. Although algae from habitats where the water is stationary seem to thrive in cultures without special devices for aeration, it seems beneficial and in some cases obligatory to aerate the culture solution. Even algae which thrive in non-aerated culture media are greatly accelerated in their growth when aeration is employed. This may be arranged either by passing a stream of ordinary air through the solution to maintain a normal concentration of dissolved gases, or by fortifying the air with about 5% carbon dioxide as a stimulant to photosynthesis. This latter practice has been widely used in physiological experiments with *Chlorella* (82, 370–373, 463) and other algae (112, 229) where it was desired to eliminate CO_2 as a limiting factor. Hollenberg (193), Rowan (417) and many others advocate the use of shallow culture vessels to secure suitable aeration. Clare and Herbst (78) aerated the culture solution after sterilization by pouring it back and forth several times into sterile containers. Papenfuss (350) found it helpful to aerate cultures of *Ectocarpus* with aspirators. Cooper (80) has recently described an ingenious and useful apparatus for growing algae from rapidly moving streams in artificial culture. This method should prove helpful with many forms re-

quiring aeration. Hollenberg (192) cultivated *Halicystis* germlings in running sea water, preventing contamination with diatoms by filtering the sea water through cotton, sand and glass wool. Bristol and Page (61) describe an excellent arrangement for aerating cultures with gases freed from bacteria.

Sterilization and storage of media. The ordinary bacteriological methods of sterilization of glassware and media are successful in the technic of algal culture. Thus, glassware is usually sterilized in a dry air oven for an hour at temperatures between 150° and 180° C. Culture media containing sugars may be sterilized either fractionally for $\frac{3}{4}$ hour in flowing steam or in an autoclave for 10 minutes at 10 pounds pressure. Subjecting certain sugars to higher temperatures and pressures often effects hydrolysis and acidification of the medium, with subsequent failure to gel. Other culture media may be safely sterilized in an autoclave for 15 minutes at 15 pounds pressure. This treatment will, however, affect media containing ammonium salts. Greathouse and Rigler (150a) have reported on the effects of various methods of sterilization on organic substances in culture media.

Sterile media in flasks and tubes may be conveniently stored in refrigerators to reduce evaporation. The latter may be further eliminated by the use of tin foil, rubber, cellophane or other specially prepared caps for the storage vessels. For ordinary purposes, media seem to remain satisfactory after indefinitely long periods of storage, but Czurda (88), in his studies of the conditions inducing sexual reproduction in *Spirogyra,* found it necessary to use freshly prepared culture media.

The pH of the culture medium. In 1896 Molisch (325) noted that his culture solution containing monobasic potassium phosphate was lethal to *Spirogyra* and other algae, but that these same organisms remained healthy if he replaced the acid phosphate with dibasic phosphate. He also suggested neutralizing acid phosphate with calcium carbonate. Richter (407) summarizes the earlier literature dealing with the reaction of the medium. Pringsheim (376), who seems to have been the first to obtain a blue-green alga in pure culture, found that these forms preferred a neutral or slightly alkaline medium, and this has been substantiated in the recent work of Allison, Hoover and Morris (8, 9). Burkholder (64) found that movement of *Oscillatoria* was most active from pH

6.4 to 9.5. Pringsheim (379) recommends a neutral or slightly
alkaline medium as favorable for most desmids. Warén (493),
however, reports that *Micrasterias rotata* grew best in media with
pH 4.7 to 5.0. Geitler (139) found it necessary to grow *Navicula
seminulum* on Knop's agar of pH 9.0 in order to secure formation
of auxospores, while Baas-Becking (20) reports a similar pH fa-
vorable to the growth of *Dunaliella*. Artari (17) states that pure
cultures of *Chlamydomonas* grow faster at first in acid media con-
taining mono-potassium phosphate, but that ultimately total growth
is the same when alkaline dibasic phosphate is substituted. Hop-
kins and Wann (198) studied especially the relation of pH to the
growth of *Chlorella* and concluded that pH 3.4 was the minimum
at which growth would occur; the maximum alkaline limit was not
ascertained. They also found that pH may produce secondary
effects, as by precipitating iron at higher values. Luksch (288),
in his paper on the physiology of the chlamydomonads, presents a
good discussion of pH, and reports the growth of various species
in pH values ranging from 3.5 to 8.6. The optimum was found to
vary considerably with the species, *Chlamydomonas pulchra* grow-
ing best at 6.2 and *C. pseudococcum* best at 8.4, for example.
Loefer (284) reviews the problem of pH in rather comprehensive
fashion and presents data obtained from growing *Chlorogonium*
in a given medium with the pH varying from 3.5 to 9.5. The mini-
mum and maximum pH values for the genus are 4.8 and 8.7, respec-
tively, and pH 7.4–7.8 is optimum. It therefore seems to be impos-
sible to generalize on optimum pH values for culture media for the
larger divisions or even genera of algae, in view of the marked
specific idiosyncrasies in this connection. The various species of
Euglena differ markedly in their pH tolerance (5, 108, 109, 163,
164, 215). Many studies of the relation of the pH to growth of
algae fail to take into account the secondary effects it may produce
in the medium, as emphasized by Hopkins and Wann (198).

If media are adjusted as to pH before sterilization, allowance
should be made for its effects, or adjustments should take place
later, using sterile-technic and -titrating solutions. The pH of the
medium changes also after sterilization, and some investigators, for
example, Damman (90), allow it to stand for as long as eight days
before inoculating. This is obviously connected with the effects of
aeration. The initial pH of the medium can be rendered acid or

alkaline by the proper choice of salts. Complete ranges of pH may be obtained by titrating with normal hydrochloric acid and normal sodium hydroxide, or by using phosphate buffers, such as were employed by Luksch (288). Rona (413) has summarized the preparation of such buffers. The initial pH of the medium is clearly modified by the growth of the organisms, either by their selective absorption of the component ions, or by their secretion products, or both. Thus, Pratt and Fong (372) have demonstrated that the medium becomes more acid when *Chlorella* absorbs ammonium ions and less acid when nitrate ions are utilized. An idea culture solution in this regard is one in which the pH value may be adjusted initially so that it remains more or less constant throughout the experimental period. Perhaps, even with algae, this ideal may be realized by the proper use of physiological buffers such as those found suitable by Trelease and Trelease (464) for use with flowering plants.

Methods of isolating algae in culture. Various expedients have been developed and recommended by investigators attempting to cultivate algae in pure and uni-algal cultures. It is difficult to discuss them in general terms and apart from the organisms for which they were devised. However, some principles are of universal application, while certain methods are of more general utility than others. Among these is the method of "plating" or "plating out," developed by Beyerinck (33) from his experience with bacteria. This is supplemented by "fishing" in which desired organisms or colonies are lifted from the plates and transferred to sterile media. Chodat (72–74) and his associates (151, 152) have successfully employed an essentially similar method, except that he recommends preliminary dilution followed by enrichment in inorganic media and the use of flasks instead of plates. Skinner (439) has published a lucid description of similar methods he employed in isolating 50 strains of soil algae. All these methods are especially valuable in isolating unicellular forms or those which have a unicellular stage; they can be applied to filamentous and membranous forms if these are broken up into minute portions by agitation or other means.

As Skinner (439) points out, the consensus of opinion among microbiologists is that it is extremely difficult to obtain pure cultures of algae. The writer obtained a similar impression from a

survey of the literature and discussion with various associates. While this undoubtedly does apply to certain forms, in general, his experience has proven quite the contrary. Given an average degree of manual dexterity, the proper culture medium, a plentiful supply of sterile vessels and implements, it is a fairly simple matter to isolate many unicellular species into bacteria-free cultures. For example, during four months of the present year the writer succeeded in obtaining 16 strains of unicellular algae in pure culture, although he devoted no more than a half day weekly to the task, once the media and glassware were prepared. He therefore feels justified in describing his methods in some detail. For general purposes, these seem to him superior in some respects to those of Chodat and of Skinner, although all are similar in principle and each investigator will undoubtedly develop modifications of his own.

Glass test tubes (media tubes) are thoroughly cleansed and filled with 5 cc. each of redistilled water or the nutrient solution to be used in the culture medium, loosely corked with rubber stoppers and sterilized in the autoclave. When cool, the stoppers should be placed firmly in position. A number of larger media tubes containing 15–25 cc. of inorganic medium solidified with 1.0 to 1.5% agar, and a similar number containing the same medium with 0.5 to 1.0% glucose added, are prepared and properly sterilized. At the same time, a suitable number of Petri dishes and 1 cc. pipettes are sterilized in a dry air oven. The writer uses Detmer's Solution (see Appendix I) in $\frac{1}{3}$ dilution for routine work. When these preliminary preparations have been completed, the procedure is as follows: the required number of tubes of media are melted and, if one is available, stored in a water bath at a temperature of 40° to 45° C. A given amount of alga-containing material (soil, liquid, *etc.*) is introduced into a sterile 5 cc. water blank, the stopper reinserted, and the tube violently shaken at least 50 times to secure as uniform as possible dispersion of the algal cells. Before settling occurs, 1 cc. of this suspension is removed to a second water blank which is then agitated like the first, and from this, 1 cc. is introduced into a third blank by a sterile pipette. One pipette may be used for a given series of dilutions. The extent of dilution of an initial inoculum must be determined by the judgment of the technician and should be based on experience; it will be determined by the concentration of algae in the original material. The follow-

ing is a more concrete example: a few drops of a grass-green liquid culture of *Chlorella* were diluted through four water blanks by the above method. This was sufficient to permit discrete growth of colonies and fishing from plates made from the third and fourth dilutions. The first two plates were discarded, having become thickly populated by bacteria and fungi. *Chlorella,* however, in the writer's experience, grows more rapidly than any other alga, and hence greater dilutions of more slowly growing algae are required, since the key to success is to have the algal colonies develop to a size sufficiently large for fishing before they are overgrown with fungi and bacteria. When the dilutions have been completed, the dilution tubes are again shaken, and 1 cc. of each is introduced carefully with a fresh sterile pipette into sterile Petri dishes properly labeled; for the third and subsequent dilutions, duplicate dishes are prepared. Then a tube of melted inorganic agar medium is removed from the water bath, the plug removed, the mouth flamed, and the contents poured into a dish containing the diluted sample, the dish being rotated to secure thorough mixing of the agar and liquid. All the dishes are treated in similar fashion; the duplicates of the higher dilutions are mixed with agar containing the same medium, enriched with 0.5 to 1.0% glucose. In the writer's experience, the growth of molds and bacteria is too rapid to make glucose plates useful in dilutions less than the third. Both kinds of agar are employed because that containing glucose accelerates growth of a number of algae, making it possible to fish colonies earlier than on the purely inorganic plates.

The plates are then placed, cover-side down (in order to prevent the water of condensation from precipitating on the surface of the agar) in a glass cabinet illuminated with a battery of 60-watt bulbs and equipped with a humidifier. Some algae form microscopically recognizable colonies within a few days to a week in Detmer's glucose agar, while others require two or three weeks. It is often necessary to discard plates made from the lower dilutions of the latter forms. When colonies suitable for fishing have developed, a number of Petri dishes containing sterile inorganic- and glucose-Detmer's agar are prepared and the bottoms marked with 6 or 8 circles with a glass marking pencil. The plate with the developing colonies is examined with an ordinary microscope at a magnification of 100 ×, and when suitable colonies are located, the

plate is transferred to the stage of a dissecting binocular. The latter is set up with transmitted light and arranged to give a magnification of approximately 28. In an earlier paper, the writer (430) recommended glass needles for transferring colonies; these have the advantage of rigidity and can be drawn out into extremely fine points. However, they are easily broken and can not be sterilized in the flame. At present, he is using platinum wire (containing 5% iridium for rigidity) Brown and Sharp gauge 32, which is sufficiently fine and withstands sterilization in a flame. With a little practice it is fairly easy to remove whole colonies or parts and transfer them to the surface of sterile agar within the marked circles. Usually, quadruplicate transfers are made, two to organic and two to inorganic media. The latter is used again at this point so that should the transfers inadvertently contain bacteria and fungi, although those to glucose agar may be unsuccessful, the algae will not be crowded out by fungi and bacteria on the inorganic agar. Should the new colonies on glucose agar seem to be free from contaminating organisms, they may be readily transferred to the same medium in flasks and stored for long periods. The purity of the cultures can be readily confirmed from time to time by inoculation into sterile bacteriological bouillon or 0.25% meat extract solution. If bacteria and molds are present, these will soon render the medium turbid. Final confirmation may be obtained by preparing smears stained with bacteriological methods.

Skinner's (439) method for soil algae is essentially similar but differs in that he employs 10 dilutions for a 10% soil suspension (using a few drops of the suspension in the first dilution), making successive dilutions in tubes of melted agar rather than water. These tubes are not poured into Petri dishes but incubated for a month or more where they obtain a few hours of direct sunlight daily. Tubes in which isolated green colonies develop are broken, the agar placed on sterile paper from which suitable colonies are transferred aseptically into similar tubes. The writer prefers his own method because it obviates breakage of tubes and especially because it should be more reliable in permitting the development of a greater variety of algae, since in the glucose medium many forms appear within a few days to a week; these may be transferred before they are overgrown with bacteria and fungi. Since Skinner stores his tubes for a month or more, many forms may be

lost, due to growth of fungi and bacteria in that period, unless, fortuitously, only algae were introduced into a given tube. The disadvantage of the Chodat method, in the writer's opinion, lies in its use of flasks instead of Petri dishes; here again it is difficult to transfer colonies early enough in their development in case molds and bacteria threaten, and it is inconvenient to spot colonies with the microscope.

Pringsheim (383) discusses methods of isolation and recommends plating out. He also advocates the bacteriological expedient of streak cultures. For this purpose, rather firm (2.0 to 2.5%) agar is desirable. Sterile Petri dishes containing about 20 cc. of medium are poured and allowed to stand until the medium solidifies. Then a platinum or chromel loop, or, better still, a glass rod with rounded end, is dipped into the alga-containing material and streaked five or six times in parallel fashion across the surface of one or more plates. In this way, as the rod moves over the agar, single cells or groups of cells are left behind at progressively greater distances in the later "streaks" so that upon incubation discrete colonies may develop. In the writer's experience, this is not so satisfactory as the plating method, because bacteria tend to spread abundantly over the surface of the agar. However, Lefevre (269) has successfully employed it among other methods in isolating desmids. As a preliminary to plating methods, bacteria and fungi, if many are present, may be greatly reduced by repeated centrifugings and decantings in sterile media, as Mainx (296) performed in isolating *Euglena*.

Obviously, the most direct method for obtaining pure cultures would be to pick up a single organism and to transfer it to a sterile culture medium, thereby eliminating other organisms at a single step. Two factors limit the utility of this method, namely, size of the organism and the tenacity with which bacteria and fungi adhere to its surface. Thus, organisms below a certain size can not be conveniently picked up under the usual magnifications of a binocular dissecting microscope, and performing such an operation under even the lowest magnifications of a compound microscope is difficult, at least for the writer. In the second place, many organisms possess a more or less viscous surface layer which harbors bacteria and fungi. Furthermore, in isolating single organisms in a vessel, the disputed question of allelocatalytic effects may be significant.

Several possibilities exist for circumventing the disadvantage of small size of the desired organism. With single zoospores or other reproductive cells which eventually grow into larger plants, one can simply distribute a number in the proper nutrient medium, and then locate single cells, awaiting such a time as they have developed sufficiently in size so as to be easily transferred. Klebs (234) employed this method for isolating *Protosiphon,* while Mainx (300) obtained pure cultures of *Oedogonium* in a similar manner. Concerning those organisms which are permanently unicellular or minute, the same methods are serviceable, since single cells on agar may be spotted and marked, their development into colonies followed through the bottom of the Petri dish with a 16 mm. objective, and they can be transferred when the colony has attained sufficient size. Minute organisms can of course be isolated by the Chambers micro-manipulator (188, 189), provided one is available and the investigator can develop proficiency in its manipulation. Hildebrand (188) has recently described methods for isolating single micro-organisms. His methods are more useful for fungi and bacteria than for algae, since in the main they depend on a growth rate which exceeds that of most algae, including *Chlorella.*

In attempting to cultivate organisms which may be readily isolated at magnifications obtainable with dissecting binoculars, the cells may be isolated from liquid or agar. In the former case, some sort of capillary pipette is necessary. This may be conveniently prepared by drawing out fine glass tubing in a Bunsen flame and subsequently drawing out still finer portions in a microflame emanating from a pointed glass tube. Grossman (157), Gross (154, 155) and Andresen (11) isolated single micro-organisms from liquids by means of such capillary pipettes. The writer finds them useful for isolating many organisms. Klugh (235) describes a useful instrument for removing single microorganisms from liquid. In the writer's experience (43), it is also easy to pick up single cells or colonies from agar media, as Alvik (10) has suggested. Rather firm (3.0%) agar is prepared and poured into Petri dishes to solidify. Liquid containing the desired organisms is then spread over the agar. The writer allows the dish to remain uncovered until the excess liquid evaporates or is taken up by the agar, so that the organisms become stranded on the surface of the latter. It is then a simple

matter to manipulate a fine glass or wire needle so as to segregate individual cells or colonies and lift them out of the agar into sterile media. It is often helpful to dip the needle into sterile agar first, so that a little adheres; this makes it easier to pick up a given cell and also precludes the possibility of its drying during transfer.

Pringsheim (391) has described in detail his methods of isolating flagellates and obtaining mono-cell or clonal cultures. His directions are too extensive to be summarized here, but they depend on the preparation of sterile fine-bore pipettes into which a number of the desired organisms is drawn by capillarity. These are transferred through a series of washings in sterile solutions and finally transplanted into sterile culture vessels. Isolation and transfer are executed in watch glasses within Petri dishes, and the manipulations are carried on aseptically. Moewus (323) has recently outlined his methods for isolating large numbers of individual *Chlamydomonas* cells. He isolates the motile products of germinating zygotes in Esmarch dishes with firm (3.0 to 5.0%) agar flooded with a thin film of liquid and stores them in the dark. As a result of this treatment, the motile zoospores escape from the zygote vesicle and distribute themselves through the liquid. By this time the latter is absorbed by the agar and the motile cells become stranded on it. Usually, well separated colonies develop from each cell when the culture dish is transferred to the light.

It is not always possible to remove bacteria from the surface of the desired organism. Thus, the presence of a gelatinous sheath in many Myxophyceae is directly responsible for the great difficulty with which such organisms are isolated, and special methods must be resorted to. However, it is possible by transferring other forms through several drops of sterile media, to "rinse" off bacteria or fungus spores. Hygen (204) obtained bacteria-free cultures of *Nematocystus* in this way. It is still more readily accomplished in cultivating motile organisms. In this case the methods used for obtaining pure cultures of *Paramecium* are helpful (176, 353, 365, 366). Hargitt and Fray (176) have shown by bacteriological methods that an individual *Paramecium,* passed through four or five rinsings in sterile media, is usually bacteria-free.

With motile algae or those with motile stages, many investigators (41, 42, 123, 184, 264, 274, 297, 300, 317, 321, 434, 449) have

devised a simple method of isolating the desired organism by taking advantage of its phototactic responses. Føyn (123) calls this method "tactic purification." A variety of procedures suggest themselves: the writer has found the following especially useful in isolating *Protosiphon* gametes and certain Volvocales. Six or more sterile Petri dishes containing sterile distilled water or dilute nutrient solution are placed on black paper and covered with blackened Petri dish covers of larger diameter. The latter have been previously thoroughly blackened with opaque black paint, except for a $\frac{1}{4}$ inch slot on the margin. A brilliant light source is set up in unilateral relationship to the row of Petri dishes so that the latter when covered are illuminated at only one point. The motile cells or those which give rise to them are cautiously introduced at the darkened side of the first dish without agitating the liquid. Preliminary experiments indicate the time required for motile cells, provided they are positively phototactic, to reach the illuminated portion of the Petri dish. Fungus spores and bacteria remain behind. When the motile cells have accumulated at the lighted spot, they can be carefully removed with a sterile pipette and transferred to the darkened portion of a second dish and again removed with a fresh sterile pipette after they have reached the lighted side. Experience demonstrates the number of times this procedure must be repeated. Finally, when it seems probable that bacteria and fungi have been eliminated, the motile cells can be introduced into the culture vessels. This method has been used extensively in obtaining pure cultures of marine Phaeophyceae and Chlorophyceae.

Several miscellaneous methods have been described which may well be of more general value. Zumstein (503) long ago relied on chemical means to reduce the bacterial contamination in his cultures of *Euglena gracilis* by acidifying the medium with citric acid. Trelease and Selsam (463) exposed *Chlorella* cultures containing protozoa to 0.45 M magnesium sulphate for 12 to 24 hours. The *Chlorella* survived but the protozoa were successfully eliminated by this treatment. Allison and Morris (8, 9) resorted to exposing bacteria-containing cultures of Myxophyceae to ultra-violet rays. Since the bacteria succumbed to this treatment before the algae, pure cultures of the latter were realized. Other methods of isolation are outlined below under the special discussion of each group.

UTILIZATION OF ALGAL CULTURES IN RESEARCH

Since they were first obtained, algal cultures have been used in a variety of morphological and physiological researches. The difficulty with which taxonomists distinguish certain species and genera of unicellular algae in mixed collections, stimulated attempts to segregate them into at least uni-algal cultures (210, 443). The doctrine of polymorphism has been carefully investigated by means of culture technic (72, 270, 276) as well as have the variability, teratology and mutation of certain forms (89, 94, 318, 343). Among the unicellular algae, *i.e.,* in genera with large numbers of species, as *Chlamydomonas, Euglena* and *Scenedesmus,* the identification of species is greatly facilitated by isolating the organisms into uni-algal cultures and comparing them with type cultures of known origin (141). While the herbarium method is as indispensable for the determination of algae as it is for higher plants, as Drouet (107) has emphasized, in such forms as euglenoids and certain Volvocales, critical characters, as the nature of flagella, structure of the chloroplast, and even cell shape (Polyblepharidaceae), are not preserved either in liquids or by drying. For identification of such forms, the availability of authentic cultures for comparison would be of inestimable value. Before the present war a number of such cultures were maintained at the Pflanzenphysiologisches Institut der deutschen Universität in Prague, and at the Botanical Laboratory of the University of Geneva. Obviously, the maintenance of such an herbarium of living algae involves expense and labor, but analogous ventures on the part of bacteriologists and mycologists seem to be successful.

Lichen taxonomy is becoming increasingly concerned with cultural studies involving the algal component of the symbiosis (209, 224, 441), and Jaag (210) has recently discussed the utility of the algal biont as a criterion in the taxonomy of the group.

The use of pure or uni-algal cultures has made possible the enormous progress which has occurred during the past 15 years in our knowledge of life cycles in the algae (173, 183, 204, 217, 223, 237, 245, 256, 262, 264, 265, 321, 349, 419). Life cycles based merely on cytological methods and material collected in nature are at best logical conjectures, unless they are supported by careful experimental repetition of the life cycle in artificial culture. Ramanathan's (400) recent paper on *Enteromorpha* is an eminent

example of the fruitfulness of culture methods. The latter have also made possible a number of important contributions dealing with sexuality and hybridization of algae, such as those of Moewus (317, 319–323), Czurda (86, 88) and many others cited in connection with life cycle studies.

Culture methods have revealed a previously unsuspected subterranean algal flora (54, 55–57, 60, 120, 195, 205, 216, 232, 254, 265, 287, 361–363, 404, 409, 438, 448, 458, 486). Algae are present in soils of all sorts, even those of the Sahara desert (232). Petersen (363) has recently reviewed the literature dealing especially with soil algae, and Waksman (486) discusses their possible rôle in the soil. Oettli's (340) report that species of *Ankistrodesmus* can utilize cellulose as a source of carbon is of interest in this connection. Pure culture studies of certain Myxophyceae, as noted above, indicate their capacity to fix free nitrogen in the soil. Engle and McMurtrey (117) have reported that algae stimulate the growth of tobacco plants in water cultures, apparently by effecting aeration.

Algal cultures have played an important rôle in physiological discoveries, especially when bacteria-free cultures were employed. The presence of bacteria introduces a source of error into otherwise carefully controlled experiments. The first physiological studies using algal cultures were in the field of plant nutrition (118), and these have continued until the present day (112, 372, 468). The recent paper of Hall and Schoenborn (169), in which they report the establishment of a photoautotrophic race of *Euglena gracilis* by gradual elimination of mixotrophic individuals from the population, is a triumph of pure culture technic and indicates the value of the method for fundamental physiological studies. *Chlorella, Nitzschia* and other algae in cultures have been the vehicles for important experiments and discoveries in the fields of photosynthesis and respiration (115, 116, 132, 133, 189, 196, 302, 373, 491, 492), as well as in population studies involving growth rates and inhibiting factors (172, 280, 370, 371). Much of our quantitative knowledge of photosynthesis is based upon work with *Chlorella,* using the technic devised by Warburg (491). This organism lends itself admirably to such studies. Cultures containing 20,000,000 cells per cc. may be obtained in four days as a result of proper conditions of cultivation (82). The further advantages of using *Chlorella* are

the facts that the cells are not injured by repeated centrifugation and washing, and that accurate measurement of the material is readily available through haemacytometer counts. Furthermore, it is apparently easier to control the environmental factors of microorganisms than those influencing higher green plants. The investigations of Meier (67, 306–310) on the effects of light on living plants have been carried on by means of pure cultures of algae.

Gunderson and Skinner (158) have described methods of growing algae in large quantities for physiological studies. Bruce, Knight and Parke (62) used pure cultures of various algae as controlled diets for oyster larvae. Buchsbaum and Buchsbaum (63) have used *Chlorella* successfully in animal tissue culture work. Schreiber (431) has suggested the use of pure cultures of algae as indicators of the productive capacity of sea water in connection with plankton studies, and Ström (451) and Franzew (127) have applied this method to fresh water.

Pure cultures of algae have proven convenient media for testing the effect of various chemical agents on living organisms. Pratt and Trelease (373), for example, have studied the effect of deuterium oxide on photosynthesis of uni-algal cultures of *Chlorella*. Other investigators have employed pure cultures of algae to study the action of various growth-promoting substances and vitamines (51, 114, 346). The report that orange juice stimulates the development of *Laminaria* gametophytes (66), emanating from England as it does, is perhaps too far afield to be considered propaganda on the part of citrus growers. The conclusion that low concentrations of ethyl alcohol stimulate growth of motile algae (286) might conceivably be used as advertising material in certain enterprises.

SPECIAL METHODS

Myxophyceae. Compared with other groups, relatively few Myxophyceae have been grown in pure or even uni-algal cultures. This is correlated with their possession of a pectin sheath which harbors bacteria and fungi. Most successful cultures have been achieved by plating out material after violent agitation so that akinetes and hormogonia become separated from the more gelatinous portions of the plant body. Germinating akinetes produce germlings which are free from bacteria. Pringsheim (376), who was the first to obtain bacteria-free cultures of Myxophyceae, re-

sorted to repeated transfers and the use of silica gel. There is practically unanimous agreement that Myxophyceae prefer neutral or slightly alkaline culture media, and most formulae include dipotassium phosphate for this reason. Delarge (98) reports that a modified Detmer's Solution at pH 6.5 with dibasic potassium phosphate substituted for the monobasic, and diluted to 1/3, is optimum for the growth of *Phormidium*. The writer has used this solution successfully for other forms.

Chlorophyceae. Probably more Chlorophyceae have been grown in laboratory culture than any other group, as indicated in Appendix II. Green algae are so diverse in habit that they can not be discussed collectively. In general, the Volvocales seem to prefer solutions of dilute concentration, such as 0.05% Beyerinck's and Knop's (43, 181). Dilute solutions effect prolonged motility. As a group, these organisms are probably mixotrophic in their nutrition (167, 283), since they thrive best in media containing substances like peptone, meat extract, *etc.* It is inconvenient to supply these substances except in pure cultures. This preference of Volvocales for environments containing organic matter long ago suggested the method of "Faulkultur" or putrifying cultures for rearing them in the laboratory (28, 29, 44, 135, 212, 320, 436). Behlau (28), for example, secured thriving cultures of *Chlamydobotrys* by using sterile covered glass cylinders (17 × 4.5 cm.) at the bottom of which he placed 6 to 9 grams of powdered egg albumin covered with several centimeters of sterile garden soil topped with a thin layer of sterile sand. The inoculum (soil, sludge, *etc.*) is placed on the surface of the sand, and water is added gently until it reaches almost to the top. The cylinder is then stored in diffuse light. Layers of bacteria arise from the soil, and finally, if successful, the water becomes green with algae. Various modifications have been suggested: instead of albumin, Moewus (320) advocates peptone, Bolte (44) uses only 0.75 grams of albumin, and Jacobsen (212) uses blood-fibrin, casein or gluten. The bacterial activity makes available organic substances which stimulate growth of the algae; the same principle is involved in Brandwein's (49, 50) method of growing *Spirogyra*. The present writer has previously discussed at some length methods for growing Volvocales, so that repetition seems unwarranted (43). Aside from *Dunaliella,* none of the polyblepharids seems to have been

cultivated in artificial culture. Moewus (316) has summarized the literature of the Volvocales up to 1931; this list undoubtedly contains citations dealing with culture methods.

The Chlorococcales grow readily in artificial culture and in a variety of media. Bacteria-free cultures of Ulotrichales and Oedogoniales are generally obtained by securing and cultivating zoospores; the young germlings are subsequently transferred and, if necessary, rinsed to free them from foreign organisms. The filamentous Zygnematales are among the most difficult organisms to obtain in bacteria-free condition because of their gelatinous surface layers and the absence of motile cells; Czurda (85) has published a detailed account of the methods by which he was successful. The simple method of cultivating *Spirogyra* permanently in the laboratory, described by Brandwein (49, 50), is a great boon to teachers who are often hard put to find this genus at certain times of the year. His methods are outlined in Appendix I. Additional citations dealing with Chlorophyceae are presented in Appendix II.

The Euglenaphyceae have been extensively cultivated in a variety of media, in mixed, uni-algal, pure and mono-cell cultures. In the writer's experience, the large species of *Euglena* with ornamented periplasts are more difficult to cultivate than the smaller forms. Gross cultures of the latter thrive in water with a small piece of decaying egg albumin. Pure cultures may be maintained in Jahn's (215) medium. Zumstein's (503) Solution seems to be far too acid for most species. The writer has cultivated certain species of *Phacus, Trachelomonas* and *Euglena* successfully in soil solutions (see Appendix I). Mainx (296) grew *Euglena* species in a similar solution.

Phaeophyceae. Next to the Chlorophyceae, the Phaeophyceae seem to have been most extensively cultivated, as indicated by the data in Appendix II. The works of Savaugeau, as listed by Dangeard (92), report the cultivation of too many forms to be included in the present paper or Appendix II. A few investigators have been successful using natural sea water. Others, like Hartge (179) and Kylin (264, 265), enrich the sea water with minute amounts of sodium phosphate and potassium nitrate. The "Erdschreiber" solution of Føyn (123) has been widely used in life cycle studies of marine algae; the soil decoction in this medium acts as a strong stimulant to growth.

Diatoms. Allen and Nelson's (7) modification of Miquel's (315) solution has been widely used in cultivating marine diatoms. A few investigators have cultivated fresh water forms (83, 139, 333). Geitler (139) was successful in cultivating *Navicula seminullum* in .5% Knop's Solution, with the pH adjusted to 8.0.

Dinoflagellates. Barker (26) states that up to 1935 only six dinoflagellates had been grown in artificial culture. He obtained bacteria-free cultures of 12 or more marine species by picking up single individuals with a Pasteur pipette. He used Allen and Nelson's (7) modified Miquel's Solution but reports that plating methods were not successful. Diwald (102) used bacteriologically filtered lake water enriched with nitrates and phosphates for growing fresh water species of dinoflagellates.

Rhodophyceae. The most neglected group from the standpoint of artificial cultivation is that of the Rhodophyceae (Appendix II). Kylin (263) used sea water with 0.2% potassium nitrate to obtain spore germination and juvenile stages of certain marine forms. Rosenberg (415, 416) has secured spore germination in certain fresh water species.

Special Difficulties. In cultivating algae, either for life cycle researches or to demonstrate certain stages, it is often difficult to obtain gamete formation, gametic union or germination of the zygote. Numerous papers (88, 102, 123, 139, 234, 272, 300, 334, 379, 395, 417, 430, 449, 499) contain helpful information on this subject. Klebs' work (234) of 40 years ago strongly implied that algae and fungi are plastic organisms whose sexual and asexual reproductive phases could largely be controlled or induced at any desired time by suitable changes in the environment. While environmental factors undoubtedly influence the initiation of reproductive phases in the life cycle, the careful work of Czurda (88) on the Zygnematales and of Geitler (139) on the pennate diatoms, indicates that certain internal conditions must also obtain before environmental stimuli became effective. The papers just cited contain suggestions for the manipulation of the environment, which, provided internal conditions are suitable, will evoke sexual reproduction.

Finally, one of the stumbling blocks to studies of sexuality and the life cycle is the difficulty of obtaining germination of those zygotes which enter into a "resting stage." Several methods have

been used to induce the germination of such dormant zygotes (76, 102, 161, 272, 368, 417, 430, 449). The most frequently recommended expedient is to dry them, either gradually or rapidly, for greater or lesser periods, at ordinary temperatures or even in the drying oven at from 40° to 60° C. When zygotes are placed in water or fresh nutrient solution after such treatments, a certain percentage usually germinate. Gussewa (161), however, was not successful with the zygotes of *Oedogonium* until they were placed in small sacs immersed in water of the natural habitat. He is of the opinion that bacteria in natural waters cooperate in germination by digesting the thick zygospore wall. Pocock (368) secured germination of *Volvox* oospores five or six months after their formation. She concludes that direct sunlight and a period of after-ripening are necessary pre-requisites; drying is not essential but beneficial. Rowan (417) has secured some evidence that certain chemical agents, indole-acetic acid, strychnine and arsenic (0.0001 to 0.0004%), for instance, stimulate germination of the zygospore in *Hydrodictyon*. Recently, Moewus (323) described a new type of soil solution, prepared without heat, which effects a germination rate of 98–99% in *Chlamydomonas* zygospores. Apparently, some stimulating ingredient of the soil is destroyed by the heat employed in preparing ordinary soil decoctions.

APPENDIX I—SOME FREQUENTLY EMPLOYED CULTURE MEDIA

It is quite impossible to list in the present paper all the diverse culture solutions which have been devised for cultivating algae. The reviewer has chosen those of most general value. Appendix II lists literature citations which provide information on special methods and particular organisms. The chemist and the plant physiologist may disapprove of the present method of writing the various formulae in terms of grams and percentages, but the writer finds this manner of presentation in the great majority of phycological papers. For morphological and qualitative work, solutions compounded according to the directions given below will be perfectly satisfactory. For careful quantitative and physiological studies, investigators may find it convenient to translate the present formulae into terms of molecular solutions, as Trelease and Selsam (463) have done. Since the indicated salts are always added to a given amount of distilled water and not "made up" to the

required amount of solution, the values (percentages) should be considered approximate. Since the quantities of each required salt in any formula are adequate and probably not limiting, the use of anhydrous salts seems unnecessary. In most cases the original formulae fail to mention water of crystallization. This probably should not be interpreted to mean that anhydrous salts were used.

I. Knop's Solution, 1865.   (See 239–241, 462)

The solutions ascribed to Knop are very diverse as to both salt content and concentration. If failure to cite it is any indication, very few of the many phycologists using so-called Knop Solution are acquainted with the source of the formula. Knop apparently developed it as a result of many experiments with various sources of the necessary ions and the effect of various concentrations on the growth of higher plants. Tottingham (462) has made an elaborate study of this solution as it affects the nutrition of the wheat plant. His paper also contains valuable historical discussions of earlier work on mineral nutrition, as well as directions for preparing the solution properly. He has, furthermore, translated Knop's formula into modern form.

Knop published the most generally used formula in at least three different places (239–241); it requires calcium nitrate, potassium phosphate (either mono- or di-basic), potassium nitrate and magnesium sulphate; traces of iron phosphate are suggested as desirable. In the writer's opinion, solutions having other salt ingredients, with the possible exception of iron, should not be designated as Knop's Solution, as, for example, is done in the Benecke-Jost "Pflanzenphysiologie" (page 135) where KCl has been substituted for KNO_3 (32). This is, in fact, a form of Detmer's Solution, as will be noted below. The writer follows Tottingham's (462) directions for preparing Knop's Solution with minor modifications. The procedure is as follows:

Part A	Part B	
$Ca(NO_3)_2 \cdot 4H_2O$. 4.0 grams	KNO_3.	1.0 gram
Distilled H_2O. . . . .500.0 cc.	KH_2PO_4	1.0 gram
	$MgSO_4 \cdot 7H_2O$ . . .	1.0 gram
	$FeCl_3 \cdot 6H_2O$	
	(1% aqueous sol.) 1 drop	
	Distilled H_2O. . . . .500.0 cc.	

Equal parts of A and B represent a 0.7% solution by weight-volume. A concentration of 0.35% is useful for general purposes, so that the definitive medium consists of $\frac{1}{2}$ part A, $\frac{1}{2}$ part B, and

1 part of distilled water. If A is mixed with the required amount of water and B added subsequently, no precipitation occurs. Such a solution is distinctly acid (pH 5.0). Substitution of di-basic potassium phosphate (K_2HPO_4) for mono-basic results in a distinctly less acid solution, but introduces the complication of a precipitate, even in very dilute solutions. The writer is unaware of any method of avoiding this, given the salts required. Tottingham states that the precipitate is calcium phosphate, so that some calcium and phosphorus are removed from the solution. It is conceivable that as the algae use the ionized calcium and phosphorus, some of the precipitate will again ionize so that no shortage of these ions occurs in the solution. In the writer's experience, the presence of the precipitate does not seem to inhibit growth of the algae. Starting with solutions containing either kind of phosphate, one can adjust the pH to any desired point by titrating with N/1 NaOH or N/1 HCl, or by using KOH and H_3PO_4 if it is desirable not to introduce new ions. Many papers fail to specify which type of phosphate was used in compounding the Knop's Solution employed; in such cases it is safest to use KH_2PO_4 and to adjust the pH after experimentation. The writer has substituted $FeCl_3$ for Knop's iron source (iron phosphate), since it is more readily soluble.

II. Beyerinck's Solution, 1898. (See 34)

As noted in the text, this widely used solution has frequently erroneously been called Benecke's Solution. The formula is as follows:

Distilled H_2O	100.0 cc.	$MgSO_4 \cdot 7H_2O$	0.02 gram
NH_4NO_3	0.05 gram	$CaCl_2 \cdot 2H_2O$	0.01 gram
K_2HPO_4	0.02 gram		

Beyerinck (34) does not make clear which kind of phosphate he employed. It is customary to add the di-basic form and, in addition, 1 drop of 1% aqueous $FeCl_3$ per liter of solution. The concentration of the solution is 0.1% by weight-volume. A 0.05% solution has proven satisfactory for many algae. The pH of a 0.1% solution is 7.2.

III. Detmer's Solution, 1888. (See 100, page 4.)

$Ca(NO_3)_2$	1.0 gram	KH_2PO_4	0.25 gram
KCl	0.25 gram	Distilled H_2O	1 liter
$MgSO_4$	0.25 gram		

"a few drops of $FeCl_3$ solution."

This solution has been widely used by Chodat and his students in a "one-third dilution." The writer uses one part of the above

solution to two of distilled water. Detmer is no more definite as to the amount of $FeCl_3$ than indicated above. The author adds one drop of a 1% aqueous solution per liter of the diluted solution. The pH of the latter is 6.2. Detmer (100), gives no source or authority for the solution, thus implying that it was original with him. However, in the fourth German edition of the same work (101) he designates the solution as "eine geeignete Nahrstofflösung (nach Knop)," thus suggesting that it is one of the many devised by that investigator. Obviously, it differs from the classical Knop's Solution in the substitution of KCl for KNO_3.

IV. Allen and Nelson's Solution, 1910.   (See 7,315,337)

Solution A	Solution B
KNO_3 .... 20.2 grams	$Na_2HPO_4 \cdot 12H_2O$. 4.0 grams
Distilled H_2O .....100.0 cc.	$CaCl_2 \cdot 6H_2O$ ...... 4.0 grams
	$FeCl_3$ (melted) .... 2.0 cc.
	HCl-pure, conc. .... 2.0 cc.
	Distilled H_2O ......80.0 cc.

To each liter of sea water are added 2 cc. of solution A and 1 cc. of solution B. "Sterilization" involves heating to 70° C. After cooling, the clear liquid is decanted from the precipitate. Galts-off (337) discusses the preparation of this solution.

V. Bristol's Solution, 1919.   (See 53–56)

KH_2PO_4	1.0 gram	NaCl	0.1 gram
$NaNO_3$	1.0 gram	$FeCl_3$	0.01 gram
$CaCl_2$	0.1 gram	Distilled H_2O ..1000.0 cc.	
$MgSO_4$	0.3 gram		

VI. Soil-Schreiber Solution ("Erdschreiber"), 1934.   (See 123, page 7)

$NaNO_3$	0.1 gram	Soil extract	50.0 grams
Na_2HPO_4	0.02 gram	Sea H_2O ......1000.0 cc.	

This is Føyn's modification of Schreiber's (431) Solution, now more widely used than the original for the cultivation of marine algae. It is undoubtedly the most valuable solution available for cultivating marine species. To prepare the soil extract, 1 kilogram of garden soil is cooked for 1 hour in the autoclave with an equal weight of distilled water. When cool, the liquid is decanted and stored in the refrigerator until needed. This modification differs from the original Schreiber's Solution in that it is slightly more dilute and contains the soil decoction.

VII. Jahn's Solution, 1931. (See 215)

KNO_3	0.5 gram	$FeCl_3$	0.05 gram
KH_2PO_4	0.5 gram	Casein (partly	
$MgSO_4$	0.25 gram	hydrolized) ..	5.0 grams
NaCl	0.1 gram	Distilled H_2O ..1000.0 cc.	

VIII. Kolkwitz' Solution, 1922. (See 243)

KNO_3	0.57 gram	$FeCl_3$	0.003 gram
$CaSO_4$	0.2 gram	Distilled (or	
KH_2PO_4	0.14 gram	Tap) H_2O ...1000.0 cc.	
$MgSO_4$	0.09 gram		

This solution is widely used in Europe, since it can be quickly made by dissolving a prepared "Kolkwitz tablet" in the proper amount of distilled or tap water. The writer has been unable to obtain the tablets in this country. They were originally manufactured by Paul Altmann, Berlin, N. W., Luisenstrasse 47.

IX. Lefevre's Solution, 1937. (See 269)

KNO_3	0.2 gram	$Ca(NO_3)_2$ .....0.1 to 0.4 gram	
K_2HPO_4	0.4 gram	Fe_2Cl_6 (Codex) ..	1 drop
$MgSO_4$ ......0.2 to 0.3 gram		Distilled H_2O .....1000.0 cc.	

This solution, for desmids, should not be confused with the Lefevre's Solution cited by Delaporte (97) for Myxophyceae. The iron ingredient is puzzling; in view of the minute amounts of iron required by most algae, probably a drop of a 1% aqueous solution of $FeCl_3$ would be sufficient. Lefevre recommends sterilization by flowing steam and adjustment of the pH to 6.0.

X. Miscellaneous Soil Extracts or Decoctions.

A. Extracts obtained with heat.

A number of methods of preparation of soil extract have been suggested which differ only in minor details. It is sometimes used as *Volvox* Solution, possibly because it was successfully employed by Mainx (297, 298) in cultivating that organism. Mainx himself refers to an earlier paper (295) for the method of preparation. There he recommends cooking one part of garden soil with one part of tap water for one hour; this is followed by filtration and dilution to 1/6 of the original strength. In another paper (296) he makes it clear that cooking should be done in the autoclave following which the liquid should be treated with ether (as a preservative) and allowed to settle for several days. For use it should be diluted with 4 to 6 parts of distilled water and sterilized, during which process the ether will be given off. Pringsheim (388) recommends

dilution from 10 to 25 times.   The writer (43) has used 1 part of garden soil to 2 of distilled water and autoclaves at 15 lbs. pressure for two hours.   This decoction is cooled, decanted, filtered several times and resterilized.   This "stock soil solution" is of general value for cultivating many Chlorophyceae when diluted as follows (either 1 or 2):

(1) Distilled H_2O ... 94.0 cc.   (2) Distilled H_2O .. 84.0 cc.
      "Stock soil solu-   "Stock soil solu-
          tion" .... 5.0 cc.   tion" .... 15.0 cc.
      KNO_3 (5% aque- KNO_3 (5% aque-
          ous solution) .. 1.0 cc.   ous solution) .. 1.0 cc.

B. Extracts obtained without heat.
Moewus' Cold Soil Solution, 1940.   (See 323)
Moewus states that this solution is especially valuable in securing high percentages of germination in the zygotes of *Chlamydomonas* species.

                                   Double-distilled
Garden soil .... 1000.0 grams H_2O ...... 1000.0 cc.

The water and soil should be mixed and allowed to stand for a week in a refrigerator, during which daily stirring should take place.   The liquid is then filtered through a membrane filter with a pore diameter of 0.75 micron to remove micro-organisms.   The filtrate is diluted 1:10 for use, presumably with double-distilled water.

XI. Brandwein's Solutions, 1940–1941.   (See 49, 50)
Brandwein's methods are reviewed here in detail as they offer a certain and simple method for maintaining adequate supplies of *Spirogyra* in the laboratory at all times.   Three liters of tap water are drawn and allowed to stand for a day.   Then 1.5 grams of hard-boiled egg yolk are triturated in a small quantity of liquid and added to the tap water.   The whole should remain uncovered for three days, whereupon *Daphnia*, preferably *D. pulex,* is added.   If these live and flourish the water is suitable for maintaining *Spirogyra*.   Cultures should remain uncovered in light of medium intensity at temperatures of 10 to 22° C.   The *Daphnia* and egg yolk may be omitted if the following solution is substituted for the tap water:

Distilled H_2O .. 0.0002 gram KNO_3 .... 0.0003 gram
Difco-Bacto $FeCl_3$ .... 0.0002 gram
    tryptophane .. 50–70 mgm.   (trace)
NaCl .... 0.003 gram $CaSO_4$ .... 0.03 gram
$MgSO_4$ .... 0.0045 gram   Distilled H_2O .. 1 liter

XII. Wettstein's Solution, 1921. (See 496)

This solution has been widely used both as liquid and solidified with 1% agar. It is often called "Wettstein's Peat Agar."
Part A.

$(NH_4)_3 PO_4$	0.2 gram	K_2HPO_4	0.05 gram
$MgSO_4$	0.05 gram	$FeCl_3(1\%)$	1 drop
$CaCl_2$	0.05 gram	Distilled H_2O	1000.0 grams

Part B.
250.0 grams of peat are cooked with 1000 cc. of water for several hours. The liquid is then diluted until it is a light coffee-brown color.
Equal parts of A. and B. make up the culture solution The ammonium salt is probably misprinted in the original as $(NH_4)_3 PO_4$ instead of $(NH_4)_2HPO_4$ or $(NH_4)H_2PO_4$.

XIII. Craig and Trelease's Solution for *Chlorella,* 1937. (See 82)

This is a modified Warburg Solution, which with light of 5,000 to 10,000 lux and aerated with 5% CO_2 in air, gives about 20 million cells per cc. in 4 days or a maximum population of 100 to 150 million cells per cc. in 2 weeks. It is well adapted to rapid culture for physiological experiments.

KNO_3	7.6 grams	$FeSO_47H_2O$	8	mgm.
$MgSO_47H_2O$	14.8 grams	*Minor-element		
KH_2PO_4	7.4 grams	stock solution	10	cc.
Potassium		Distilled water	1000	cc.
citrate	8 mgm.			

* The minor-element stock solution contains, dissolved in 1000 cc. of distilled water: 100 mgm. $ZnSO_47H_2O$; 100 mgm. H_3BO_3 ; 150 mgm. $MnSO_44H_2O$; 3 mgm. $CuSO_45H_2O$.

For use mix 1 part of the concentrated solution with 2 parts of distilled water. Growth is reduced only about 15% by mixing 1 part of the concentrated solution with 15 parts of distilled water.

APPENDIX II—CITATIONS OF PAPERS INVOLVING THE CULTIVATION OF VARIOUS GENERA OF ALGAE

This summary includes mainly papers published from 1927 to the present, but certain earlier reports are listed, either because of their importance or because they have been omitted in previous reviews. For data similar to those in the present appendix, consult 299 and 334.

MYXOPHYCEAE:

Anabaena (96, 106, 172, 201, 205, 378, 484); *Calothrix* (293, 447); *Catella* (10); *Chroococcus* (97, 172); *Cylindrospermum* (147, 293); *Gloeocapsa* (218); *Nostoc* (8, 9, 95, 97, 106, 175, 218, 293, 376, 378, 484, 498); *Oscillatoria* (64, 81, 97, 119, 293, 333, 376, 424, 454); *Phormidium* (45, 81, 96, 97, 98, 119, 172, 205, 424); *Rivularia* (218); *Schizothrix* (97); *Scytonema* (427); *Spirulina* (81); *Synechococcus* (10); *Tolypothrix* (106, 194); *Miscellaneous* (68).

CHLOROPHYCEAE:

Acetabularia (170, 171); *Anadyomene* (208); *Ankistrodesmus* (340); *Arthrodesmus* (269, 270); *Boekelovia* (338); *Brachio-monas* (338); *Bryopsis* (502); *Caespitella* (478); *Capososiphon* (37); *Carteria* (3, 10, 29, 46, 212, 288, 430); *Chaetomorpha* (183); *Chlamydobotrys* (28, 449); *Chlamydomonas* (17, 18, 29, 46, 89, 126, 141, 205, 212, 225, 261, 268, 289, 290, 317, 318, 322, 323, 344, 436, 449, 451, 475); *Chlorella* (10, 14, 15, 33, 51, 58, 61, 71, 74, 82, 103, 115, 116, 132, 133, 196, 198, 199, 308, 311, 312, 329, 357, 370–373, 411, 451, 461, 463, 468, 490, 491, 492); *Chlore-ceras* (422); *Chlorococcum* (10, 41, 43, 53, 58); *Chlorogonium* (168, 180, 212, 279, 284, 286, 297, 359, 391, 430, 449); *Chloro-sphaera* (334, 478); *Cladophora* (37, 80, 123); *Closterium* (11, 43, 269, 414); *Coccomyxa* (51, 211, 346, 451); *Codiolum* (219); *Coelastrum* (146, 157, 346, 476); *Cosmarium* (11, 88, 268, 414); *Cylindrocystis* (226); *Cystococcus* (58, 246); *Derbesia* (245); *Des-midium* (270); *Draparmaldia* (453, 469); *Dunaliella* (20, 112a, 272, 338, 359, 360); *Enteromorpha* (36, 38, 39, 183, 321, 400, 500, 500a); *Eremosphaera* (295, 327, 403, 485); *Euastrum* (268–270); *Eudorina* (40, 43, 111a, 181, 182, 297, 430); *Halicystis* (192, 245); *Haematococcus* (113, 114, 213, 290, 297, 344, 377, 436, 499); *Hormiscia* (178); *Hormidium* (196, 205, 450); *Hyalo-gonium* (391); *Hyalotheca* (11, 88, 270); *Hydrodictyon* (43, 220, 301, 347, 417); *Kentrosphaera* (403); *Kirchneriella* (10); *Lepto-sira* (478); *Lochmiopsis* (418); *Mesotaenium* (51, 84, 85, 270); *Micrasterias* (268, 269, 270, 386, 493, 494); *Microthamnion* (324, 401); *Monostroma* (37, 256, 321, 500, 500a); *Mougeotia* (137); *Muriella* (480); *Oedogonium* (129, 160, 161, 249, 300); *Oliviera* (336); *Palmelococcus* (346); *Pandorina* (40, 43, 430); *Pediastrum* (10, 348, 465); *Pleurastrum* (478); *Pleurococcus* (34, 501); *Polytoma* (135, 212, 289, 320, 380, 387, 389, 392, 394, 449); *Polytomella* (392); *Prasiola* (236); *Protococcus* (196, 490); *Protosiphon* (42, 43, 317, 319, 335, 490); *Pseudoclonium* (475); *Pseudendocloniopsis* (478); *Pseudendoclonium* (478); *Raphidonema* (478); *Pseudopleurococcus* (478); *Scenedesmus* (14, 19, 33, 58, 94, 132, 133, 157, 205, 252, 332, 336, 411, 443, 451,

468, 476, 490); *Sorastrum* (349); *Sphaerella* (358); *Sphaero-zosma* (270); *Spirogyra* (31, 49, 50, 84–88, 200); *Spondylo-morum* (212); *Spongioplastidum* (478); *Staurastrum* (270); *Stephanosphaera* (317, 436); *Stichococcus* (14, 15, 19, 67, 112, 153, 196, 205, 309, 310, 346, 411, 451, 476, 490); *Stigeoclonium* (221, 292, 402, 470); *Trentepohlia* (224, 441); *Ulothrix* (77, 134, 156, 478); *Ulva* (124, 321); *Uronema* (478); *Urospora* (219); *Valonia* (420); *Vaucheria* (173, 495); *Volvox* (238, 268, 297, 298, 368, 386, 473); *Zoochlorella* (285); *Zygnema* (84–88).

EUGLENAPHYCEAE:

Colacium (217, 296); *Euglena* (5, 23, 24, 108–111, 148, 159, 162–166, 168, 169, 203, 214, 215, 230, 250, 267, 268, 289, 294, 296, 344, 375, 456–458, 503); *Eutreptia* (26); *Lepocinclis* (268); *Phacus* (250, 268, 296); *Trachelomonas* (143).

HETEROKONTAE:

Botrydiopsis (19, 138); *Botrydium* (314, 482); *Brachymenia* (10); *Bummileriopsis* (480); *Characiopsis* (369); *Chlorellidium* (481); *Chlorobotrys* (138, 369); *Dictyococcus* (480); *Goni-ochloris* (138); *Heterococcus* (369, 480, 481); *Heterothrix* (480); *Mischococcus* (477); *Ophiocytium* (138); *Tribonema* (138, 186, 369).

DINOFLAGELLATAE:

Ceratium (26); *Exuviella* (26); *Glenodinium* (102, 242, 274, 403); *Gonyaulax* (26); *Gymnodinium* (274); *Gyrodinium* (274); *Peridinium* (26, 274); *Prorocentrum* (26, 154).

BACILLAREAE:

Amphipleura (260); *Biddulphia* (154, 431); *Chaeroceras* (154, 431); *Coscinodiscus* (154); *Ditylium* (154, 155); *Fragillaria* (52); *Eunotia* (139); *Gomphionema* (139); *Melosira* (154, 431, 433); *Navicula* (22, 52, 83, 139, 405); *Nitzschia* (22, 25, 52, 83, 185, 228, 229, 356, 364, 405, 445); *Pinnularia* (139, 333); *Rhizo-selenia* (154, 155); *Skeletonema* (154, 155); *Streptotheca* (154); *Synedra* (189); *Thalassiosira* (6, 154).

PHAEOPHYCEAE:

Agarum (223b); *Alaria* (222, 397); *Arthrothamnus* (222); *As-cocyclus* (265); *Asperococcus* (237, 264–266); *Castagnea* (352); *Chorda* (223, 264, 265); *Colpomenia* (257); *Costaria* (22); *Cut-leria* (425); *Desmarestia* (2, 434); *Desmotrichum* (265); *Dictyo-siphon* (125); *Dictyota* (435); *Ecklonia* (207, 223a); *Eckloniop-sis* (223a); *Elachistea* (266); *Ectocarpus* (125, 184, 265, 350); *Egregia* (331); *Eisenia* (78, 193, 223a); *Endarachne* (467);

Eudesme (125, 265, 352); *Haplospora* (90); *Heterochordaria* (1); *Himanthalia* (142); *Kjellmaniella* (223); *Laminaria* (66, 105, 177, 222, 223, 223b, 231, 262, 330, 432, 497); *Leathesia* (90, 265); *Litosiphon* (265); *Macrocystis* (48, 99, 273); *Mesogloia* (125, 265, 352); *Myriocladia* (265); *Myrionema* (266); *Myrio-trichia* (266); *Nematocystus* (204); *Nereocystis* (179); *Pela-gophycus* (187); *Phleospora* (304); *Phyllitis* (265); *Pleurophy-cus* (13); *Postelsia* (330); *Pterygophora* (305); *Punctaria* (467); *Pylaiella* (90, 266); *Ralfsia* (266); *Scytosiphon* (1, 90, 130, 265, 467); *Sorantha* (12); *Sorocarpus* (1); *Spermatochnus* (351); *Stilophora* (264, 265); *Striaria* (266); *Taonia* (412); *Tilopteris* (90); *Undaria* (222, 223a). For many more genera and species see the papers listed in 92.

RHODOPHYCEAE:

Actinococcus (69); *Asterocystis* (416); *Ceramium* (90); *Hal-arachnion* (90); *Lemanea* (415); *Nemalion* (70); *Porphyra* (91); *Porphyridium* (479).

MISCELLANEOUS:

Charophyta (483).
Chrysophyceae (62, 297).
Cryptophyceae (26, 62, 403).

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THE BOTANICAL REVIEW

SYSTEMATICS, CYTOGENETICS AND EVOLUTION IN CREPIS

ERNEST B. BABCOCK

University of California, Berkeley

CONTENTS

INTRODUCTION

Plant taxonomy exhibits a growing tendency to become more dynamic. The increasing interest on the part of plant systematists in what is coming to be known as "experimental taxonomy" and "the new systematics" is a hopeful sign. The essence of this broader approach to the problems of taxonomy is the synthesis of evidence from all available sources which may throw light on the relationships between species and larger groups of organisms. This approach may be contrasted with that of those museum workers who are interested only in pigeon-holing specimens as rapidly as possible, or with that of the "orthodox" taxonomists who have no interest in garden cultures, hybrids or other experimental evidence. This is not the place to discuss the relative importance of different categories of evidence. But the majority of those interested in making taxonomy a dynamic part of biology may agree with the recent conclusion of Smith (113): "I believe that the last word lies with the morphology. But I can record without hesitation my obligations to the cytologist. His contribution has been of the greatest interest and value." What is true of cytology may also be true, and in many cases to a greater degree, of genetics, experimental ecology and geographic distribution.

In the decade following the report on *Crepis* by Babcock and Navashin (18) in 1930, considerable progress was made in research on this genus. The cytological studies have been confined mostly to the chromosomes. The descriptive work has been done mostly by the writer and his associates (with the exception of a few new species which have been published by other authors), the ultimate goal being a systematic treatment of the genus based on all available evidence. The world monograph of the genus has not been published but it is nearing completion. Therefore, the present review will summarize the papers, published for the most part during the past decade, which have some bearing on the systematics of the genus. In addition, several papers on the cytology of *Crepis* which have at present no obvious bearing on systematics will be mentioned.

Acknowledgments

The accumulation of a living collection of over 100 species of *Crepis* from 3 continents has been a time-consuming undertaking,

requiring the collaboration of hundreds of persons. To the directors of botanical gardens, botanists, plant collectors and many others who have assisted in this work, due appreciation is extended. The investigations for which the reviewer has been responsible could not have been completed without grants-in-aid from several sources. Grateful acknowledgment is made to the Carnegie Institution of Washington, the Rockefeller Foundation, the American Philosophical Society, the Society of the Sigma Xi and the Board of Research of the University of California for grants received. To numerous Russian biologists who have carried on important researches on certain species of *Crepis,* a token of appreciation is in order, especially to those who have supplied reprints, and particularly to my former associate, Dr. M. S. Navashin, whose early and lasting enthusiastic interest in this group of plants has resulted in contributions of outstanding importance. To my former coworkers on the cytology and genetics of *Crepis,* Dr. J. L. Collins, Dr. Margaret Mann Lesley, Dr. Lillian Hollingshead Hill, Dr. D. R. Cameron and Mr. C. W. Haney, and to my present associates, Dr. G. L. Stebbins, Jr., Dr. J. A. Jenkins and Mr. Ernest Jund, my warm gratitude is expressed for loyal assistance. Finally, special acknowledgment is made to Dr. Stebbins and Dr. Jenkins for their critical reading of the manuscript of this review and for very helpful suggestions, at the same time absolving them from any responsibility for possible errors in judgment or expression.

Limitation and Relationships of Crepis

The species excluded remain essentially as proposed by Babcock and Navashin (18). The genus *Youngia* of Cassini has been revived by Babcock and Stebbins (19) and includes 27 species, most of which were transferred from *Crepis*. "The distribution of the genus (*Youngia*) taken as a whole is entirely consistent with the conception that it is a natural group which had its origin in southeastern Asia and that evolution has been accompanied by extension of the geographic range to its natural limit on the south and east and slightly beyond the great mountain barrier to the north and west." The genus *Crepidiastrum* Nakai has been combined with *Ixeris* Cass. by Stebbins (115). Those remarkable alpine species of southeastern Asia, formerly known as section Glomeratae under *Crepis,* now comprise a new genus, *Soroseris,* by Stebbins (116)

who has also revived the genus *Dubyaea* (116) of De Candolle and referred to it 3 species which have been assigned to various genera, including *Crepis, viz., C. Dubyaea* Marq. et Shaw = *C. bhotanica* Hutchinson; *C. oligocephala* Sch. Bip. sub Paleya = *C. sibirica* Clarke; and *C. tsarongensis* Anthony. Finally *Crepis glomerata* var. *porphyrea* Marq. et Shaw and *C. disciformis* Mattfeld have been referred to *Lactuca* by Stebbins (114).

CLASSIFICATION, DISTRIBUTION AND SUBDIVISION OF *Crepis*

Criteria of Systematic Classification

A general review of recent developments in taxonomic methods will not be given here. That the cytogenetic-taxonomic research on *Crepis* was one of the earliest attempts to combine all possible approaches to the classification of the species in a large and difficult genus is a matter of record (*cf.* 2). Delay in completion of the world monograph has been due, in part, to difficulty in obtaining species in living condition representing all subdivisions of the genus. Even now, living representatives are lacking for 4 of the 29 sections. Efforts at a broad and thorough attack on the problem were intensified after publication in 1930 of the comprehensive review of Rosanova (104) who covered in detail the development of plant systematic methods based on morphology, anatomy and paleontology, on phylogenetic concepts, comparative ontogeny, geographic distribution and ecology, the physiological-chemical and serological systems, and the experimental genetic and cytological methods of attack. This comprehensive treatise included a comparison of descriptive and analytical systematics and concluded with a quotation from Bateson (23) to the effect that both geneticists and systematists must gain from closer cooperation in an effort to solve the problems of evolution. An early paper of Levitsky (64) on karyotypic and genotypic transformations in the process of evolution was also stimulating to the earlier workers on *Crepis;* and another paper by Levitsky (65) defined two important terms which have been generally used in cytotaxonomic work as follows: *a*) The notion of the "karyotype" denotes a complex of nuclear characters keeping its significance now over individual, then over race, species, genus, *etc.; b*) An "idiogram" is a graphical representation of a karyotype based on chromosome morphology.

The concept of the karyotype, as used in this review, is that of

Navashin (87, p. 193), *i.e.,* the visible morphological pattern of the chromosomes. It should be emphasized that, whereas similarity in karyotype may indicate genotypical and structural similarity between 2 forms, nevertheless, it is possible for 2 forms to have similar karyotypes and at the same time to differ widely as to genic constitution and structural arrangement of the chromosomes.

In 1931 Babcock (4) pointed out that the evidence from cytogenetic research on intraspecific and interspecific variation can be combined with the basic ideas in a species-concept so as to add considerably to the definiteness of the concept, and emphasized the importance of using all possible criteria for recognition of species in taxonomic work. In 1933 Smith (113), reporting on *Primula,* a large genus which he had divided into 32 sections classified on purely morphological grounds, announced a remarkable degree of coordination between this classification and one worked out on the basis of chromosome number and the degree of uniformity or variability in the karyotypes of the species studied cytologically. But he found that certain adjustments between the two classifications brought mutually advantageous results, and he concludes that "only by marshalling *all* the data, morphological and cytological, can a final judgment be made." The comment of Darlington (32) that *Primula* and *Crepis* are exceptionally favorable material for purposes of classification on the basis of the karyotypes is undoubtedly true.

Earlier Studies on Crepis

In the earlier studies on the cyto-taxonomy of *Crepis* by Hollingshead and Babcock (53) and Babcock and Cameron (14), the evidence on chromosome number, size and shape was considered with reference to older classifications which were based on morphology alone and were all incomplete and inadequate. They could not serve, therefore, as a basis for such a test as was made with *Primula.* But, in general, it has been possible to arrange the 29 sections which have been recognized in a sequence approximating their relative degree of primitiveness or advancement. Throughout the genus there was found to be close correspondence between chromosome numbers and external morphology of the plants. The most primitive species which had been studied thoroughly had 5 as the haploid number, but there are fairly primitive 4-chromosome species. In both 5-paired and 4-paired series there is abundant

evidence of progressive development from the woody-based peren-
nial types with large, simple or lyrate-pinnatifid leaves, few large
heads, large florets and large unspecialized fruits to the short-lived
annual types with small or dissected leaves, numerous small heads,
small florets, and very small or highly specialized fruits.   The basic
primitive number was then stated to be 5, the phylogenetic rela-
tions being based primarily upon morphology of the plants, *not*
upon chromosome numbers.

Number of Species of Crepis

Since 1930, 14 new species of *Crepis* have been published by
various authors.   These, together with 18 either new or trans-
ferred from other genera, bring the total number of species recog-
nized by the present author to 195.   Of these, 113 have been exam-
ined cytologically, including 18 species which will be reported for
the first time by Babcock and Jenkins (17).   Thus, 58% of the
known species of *Crepis* have been examined cytologically, at least
as to number, size and shape of the chromosomes.

Subgeneric Classification

In previous publications on the cyto-taxonomy of *Crepis,* par-
ticularly that of Babcock and Cameron (14), it has been assumed
that the three principal subdivisions of the genus, recognized by
Bentham and Hooker in the Genera Plantarum, *viz., Catonia,
Eucrepis* and *Barkhausia,* could be treated as subgenera.   But a
more thorough study of the comparative morphology of all the spe-
cies seems to indicate that these are not truly natural or at least not
clearly delimited subdivisions.   It has been found necessary, there-
fore, as well as advantageous, to recognize numerous sections.
Whether these sections will fall completely into one of a few major
subgroups remains to be determined.   These sections will be de-
scribed and their relationship discussed in the monograph already
mentioned.   In the forthcoming report on the chromosomes, Bab-
cock and Jenkins (17), all the species which have been examined
cytologically will be listed alphabetically without reference to sec-
tions.

Geographic Distribution

The geographic distribution of 95 species of *Crepis* which had
then been examined cytologically was considered with reference to

their relationships, as indicated by their morphology and their chromosomes by Babcock (9). In general, the evidence on groups of closely related species was found to be consistent with the hypothesis that the center of origin and distribution of the genus is in southcentral or southwestern Asia and with the conclusion that 5 and 4 are the basic haploid chromosome numbers in the genus, with 5 as the more primitive number. (On the basis of later work, this conclusion must now be modified by the recognition of 6 as a still more primitive number in *Crepis,* but this does not change the hypothesis as to center of origin, although it does strengthen the hypothesis that 8 and 9 are the primitive numbers in the subtribe Crepidinae.)

Geographic isolation has been used as an important criterion, along with morphological and genetic evidence, in recent investigations by Babcock and Cave (15) and by Jenkins (55) for the recognition of species which might otherwise be treated as subspecies by some taxonomists. These studies are discussed below.

EVOLUTION OF THE KARYOTYPE IN *Crepis*

The Chromosomes of Related Genera

The earlier reports on chromosome numbers in genera close to *Crepis,* compiled by Tischler (125) and Gaiser (37), have been supplemented by Babcock, Stebbins and Jenkins (20) who reported on 9 species of *Prenanthes,* 25 of *Lactuca,* 2 of *Dubyaea,* 2 of *Youngia,* 1 of *Cephalorrhynchus* and 2 of *Ixeris.* It was concluded that 16 is probably the more primitive number in *Prenanthes, Dubyaea, Youngia* and *Lactuca;* and it was suggested that 16 and 18 are probably primitive numbers for the subtribe Crepidinae, while 10 and 8 have been derived by reduction. This has an important bearing on phyletic problems in *Crepis.* Babcock (9) had suggested that 10 is the primitive number in the Crepidinae and that the higher numbers in *Prenanthes, Lactuca* and *Hieracium* arose through hybridization among species ancestral to those genera, as well as to *Crepis,* followed by amphidiploidy. From the evidence from both the chromosomes and the plants themselves in *Crepis* and closely related genera, it now seems more likely that the earliest Crepidinae had 16 or 18 chromosomes. Chromosome morphology and meiosis have also been studied by Whitaker and Jagger (130) in 9 species of *Lactuca,* including the 2 American

species, *L. canadensis* and *L. graminifolia,* both with $n = 17$, together with F_1 and F_2 hybrids between them. The regularity of meiosis and high fertility of the F_1 hybrids support the hypothesis proposed by Babcock, Stebbins and Jenkins (20) that the American species of *Lactuca* are amphidiploids.

Chromosome Numbers in Crepis

The genus consists of 2 different groups of species on the basis of chromosome numbers alone, since the American species, excluding 2 members of an Old World section, have the base number, $x = 11$, a number which does not occur in any Old World species. These American species are discussed under polyploidy.

In the genus, as now delimited, the known haploid numbers in Old World diploid species are 3, 4, 5, 6, 7; and there are 6 polyploid species, consisting of 3 tetraploids with $x = 4$, 2 octoploids with $x = 5$, and 1 octoploid or decaploid with $x = 5$ or 4. The distribution of haploid numbers among the Old World diploid species is as follows:

Haploid number	Number of species
3	3
4	57
5	19[1]
6	14
7	3

The 3-paired species include the well known *C. capillaris, C. fuliginosa* which was first reported by Babcock and Cameron (14), and *C. Zacintha* (17), formerly *Zacintha verrucosa,* first reported under this name by Navashin (78). It is very improbable that more than 1 or 2 more species, among those now known to exist, will turn out to have 3 or less haploid chromosomes. Apparently, 3 represents an end-point in an evolutionary process resulting in reduction of chromosome number.

The 3 species with $n = 7$ have 4 close relatives that are referred to the same section because of general morphological similarity, and it is safe to predict that these will be found to have the same chromosome number. On the basis of the plant morphology, however, these 7 species are not among the most primitive sections of

[1] One of these species, *C. syriaca,* was found by Cameron (24) to have a constant 5-paired somatic complement together with 0–8 supernumerary chromosomes.

Crepis. It has been suggested by Babcock, Stebbins and Jenkins (20) that there is a close genetic connection between these 7-paired *Crepis* species and the 7-paired *Ixeris alpicola* as a result of intergeneric hybridization between the 2 genera when they were in a formative period, a suggestion compatible with the chromosome number, morphology and geographic distribution of the 2 groups.

The remaining Old World diploid species have $n = 4$, 5 or 6, with 4 predominating among the species thus far counted. This bare statistical fact has led to the false assumption by Wanscher (129) that 4 is the basic number for the genus. This assumption, also maintained by Darlington (31, p. 233), ignores the fact that nearly half of the 4-paired species are the most reduced and specialized morphologically of all the species in the genus, and that none of the remaining ones is as primitive in its morphology as some of the species with higher numbers. This had been shown by Babcock and Cameron (14) ; and it was restated by Babcock (6) that chromosome numbers alone are an inadequate criterion of relationship in *Crepis* and that the evidence on chromosome numbers must be considered in relation to the comparative morphology of the plants and the chromosomes, while geographic distribution, comparative genetics and cytogenetics also throw light on phylogeny. The 9 most primitive species in the genus, as determined from both plant morphology and chromosome morphology, are *C. geracioides*, $n = 6$; *C. kashmirica*, $n = 6$; *C. paludosa*, $n = 6$; *C. viscidula*, $n = 6$; *C. terglouensis*, $n = 6$; *C. pygmaea*, $n = 6$; *C. sibirica*, $n = 5$; *C. pontana*, $n = 5$; and *C. albida*, $n = 5$. The citation of these 5-paired species as the most primitive in the genus by Babcock and Cameron (14) was done before living plants of some of the 6-paired species were available for study and before the comparative morphology of the 6-paired karyotypes was definitely worked out. The basic, *i.e., most primitive* numbers in *Crepis,* are 6 and 5, not 4. As far as *number of species* is concerned, there are 96 diploids, of which 34% have either 5 or 6 as the haploid number and 60% have 4, the remaining 6% being the 3-paired and 7-paired species. When the chromosome numbers of all the species in the genus are known, the proportion of 4-paired species will probably be somewhat larger; but this means simply that reduction in chromosome number has accompanied differentiation, marked by morphological reduction and specialization in this genus.

It should also be made clear that at no time has a phylogenetic scheme for *Crepis* been proposed on the basis of chromosome number *alone*. Yet the following sweeping statement of Darlington (31, p. 172) would certainly give this impression: "The extensive phylogenetic conclusions that have been based on observation of pairing in hybrids, especially in *Triticum* and *Nicotiana,* must therefore be regarded as little better founded than those based on chromosome number in *Crepis*." A cursory examination of the chart of Babcock and Cameron (14, p. 292), bearing the caption "Chromosome Number and Phylogeny in *Crepis,*" might lead to the inference that the scheme there depicted was based solely on chromosome numbers. But the reading of one paragraph of the text on the same page would dispel any such notion. Even in *Primula,* for which a phylogenetic treatment was worked out by Brunn (*cf.* 113) on the basis of cytology, comparative differentiation within the karyotypes was used in addition to chromosome numbers. The statement of Darlington, quoted above, at least in so far as it applies to *Crepis,* is unwarranted and completely misleading.

Many variations from the typical diploid chromosome numbers have been reported in *Crepis.* Haploid *C. capillaris* plants were first reported by Hollingshead (*cf.* 18). They appeared among progeny obtained by crossing with *C. tectorum.* A haploid, "androgenetic" plant of *C. tectorum* was obtained by Gerassimova (43) as a result of first treating a plant having certain dominant characters with x-rays and then applying pollen from a plant with the corresponding recessive characters. The resulting haploid had only the recessive characters, and hence is assumed to contain chromosomes from the male parent only. Various polyploid forms of *C. capillaris* and *C. tectorum* have been studied by Navashin (*cf.* 18), Hollingshead (50) and Geitler (40). Notable differences in cell size in haploids and polyploids, as compared with diploids in *C. capillaris,* were observed by Hollingshead (*cf.* 18) and by Navashin (81) who also compared cell area and cell volume in 13 *Crepis* species with the average total lengths of the chromosomes in each species and found that the volume of the cell was proportional to the amount of its chromatin. Trisomic plants are not infrequently observed among extensive cultures of *Crepis.* Navashin (80) reported a trisomic *C. tectorum* plant in which the extra member

was an atypical satellited chromosome. Navashin (77) also investigated an unbalanced chimerical *tectorum* plant consisting of a normal portion and two basal trisomic shoots. Shkvarnikov (108) examined the size of the meristematic cells in the 4 possible trisomics of *C. tectorum* and found that, when either of 2 of the 4 chromosomes was present as the extra member, cell size was increased, but that the effect of either of the other 2 in the trisome was a sharp decrease in cell size, thus demonstrating the specific nature of the individual chromosomes. Trisomics and polyploids were found to occur spontaneously in *C. capillaris* and *C. tectorum* by Navashin (*cf.* 18) who examined the chromosomes of 6,000 plants. The data are summarized by Darlington (31, p. 70). All 4 of the simple trisomics of *C. tectorum* have been described by Gerassimova (46, 47) who has also discovered certain specific effects of parts of these chromosomes. Naturally occurring tetraploid and trisomic forms have been discovered among wild plants by Babcock and Cameron (14) in *Crepis vesicaria.*

Karyotypes of Crepis

The original work of Navashin (*cf.* 18) dealt with 3-paired, 4-paired and 5-paired species. For the purpose of comparison, he labeled the members of the idiograms with capital letters, a device which is still used in describing *Crepis* karyotypes. The complement of a species with $n = 5$ was found to consist of 4 pairs with a subterminal spindle-fiber attachment or centromere, while the fifth pair has a median or nearly median centromere. Of the 4 with a subterminal centromere, the 1 with the longest proximal arm was designated *A,* that with next longest proximal arm was usually *B,* that with next longest proximal arm was usually *C,* and the *D* chromosome had an extremely short proximal arm or "head" bearing a satellite; the medianly constricted member was labeled *E.* As a matter of fact, the distinctions between type *B* and type *C* and sometimes between type *B* and type *A* are often arbitrary; but in many 5-paired species it is easy to distinguish definitely between the 5 members of the karyotype. In the 4-paired species which he studied, the *E* chromosome was always lacking; and in *C. capillaris* both *E* and *B* are absent. In the form of *C. rubra* ($n = 5$) which he examined, the *C* as well as the *D* chromosome had a satellite. It should be noted that the idiogram of *C. rubra,* "Edinburgh"

strain, reported by Koller (56), can not be compared directly with Hollingshead and Babcock's (53) strains 1506 and 1176 because of the difference in the fixatives used. But the latter strains also differ in size of the satellites. Later investigations (summarized in 14 and 17; *cf.* 73 and 77) have brought to light numerous other variations in the model karyotypes described by Navashin. Also, the more primitive 6-paired species have less differentiated chromosomes, there being two or three larger pairs with a median or nearly median centromere; whereas several more advanced 6-paired species have some of the most highly differentiated chromosomes in the entire genus, as reported by Babcock and Cameron (14, pp. 312–13). It should be noted that, in the earlier years of the *Crepis* investigations, Navashin held the idea that similar chromosomes in diverse species were actually homologous but that this notion was later abandoned.

The phylogenetic significance of chromosome size and shape can be interpreted only in relation to or with aid of other criteria. In discussing the results of his experiments with hybrids between *C. capillaris* and 3 different 4-paired species, Navashin (*cf.* 18) expressed the opinion that differences in the chromosome morphology of species must indicate phylogenetic divergence, whereas resemblances would indicate close phylogenetic relationship. Geitler (39) objected to Navashin's homologizing of the chromosomes of different species on the basis of external morphology, asserting that the problems of species formation can not be solved in this way. Delaunay (*cf.* 31, p. 57) reported notable changes in length and width of the chromosomes in root tips of the same plant after subjection to lower temperature. Minor differences in size, location of the centromere, and in the satellites, including heteromorphism in a given pair of chromosomes, were reported in different strains of the same species (14, 53, 82). Notable differences in the length of the chromosomes, in species which are obviously very close, were reported in the papers just cited; also in the width (Tobgy, unpublished). That length of the chromosomes is under genotypic control, was concluded by Darlington (29) who cited, among other evidence, the report of Navashin (81) that the chromosomes of *C. capillaris* are longer and those of *C. neglecta* are shorter in an F_1 hybrid between them than in the parental species. Additional evidence was reported by Navashin (87) who found, in several

interspecific *Crepis* hybrids, the occurrence of a differential effect on the chromosome, consisting of disappearance of the satellite due to its fusion with the proximal end, a reversible process termed "differential amphiplasty," and in other hybrids a general shortening or lengthening of all the chromosomes of a specific complement, "neutral amphiplasty." In no case did the change occur in both haploid complements, but one specific group always shortened while the other lengthened. It has been assumed by Darlington (31, p. 57) that the species involved are genetically different as to control of spiralization, while the genotype of the hybrid determines a uniform width in its chromosomes whatever their width in the parent from which they are derived. A new problem is raised in this connection by the discovery of Tobgy (unpublished) that the chromosomes of *C. fuliginosa, n* = 3, are definitely narrower than those of its close relative, *C. neglecta, n* = 4, and that the narrower width of the *fuliginosa* chromosomes is constant in hybrids between the two species.

In general, after having compared the karyotype of 108 species, with reference also to the evidence on relationship provided by other criteria than the chromosomes, Babcock and Cameron (14) concluded as follows: *a*) morphologically similar species have similar chromosomes; *b*) similarity in chromosome types and in details of size and shape is an index of phylogenetic relationship (when used, of course, in connection with other criteria); *c*) both increase and decrease in chromosome size have occurred in the evolution of the genus; *d*) there is a general tendency toward reduction of size of the chromosomes concurrently with reduction in size of the plant and reduction or specialization of parts; *e*) there have been many changes in chromosome shape, as determined by relative length of the arms, and by these differences chromosomes of the same types from different species can be identified in hybrids; *f*) this fact makes it possible, by analysis of the haploid karyotype, to determine the mode of origin of certain species; *g*) chromosome number and morphology are taxonomic criteria of great value in this genus. But it must be used in connection with other available criteria such as comparative morphology and geographic distribution. Certainly, absolute identity of the chromosomes can not be set up as of paramount importance in the classification of species, for species are known in which the different forms exhibit differ-

ences in number, size or shape of the chromosomes. The genus is still evolving, and visible changes in the chromosomes are part of the process. Many alterations in specific karyotypes have been experimentally induced and some have arisen spontaneously. These are discussed below.

Chromosome Structure

Spiralization of the chromonemata and its association with chiasmata were reported in *Crepis* by J. Clausen (26; *cf.* 18) whose observations were supported by Richardson's (102). Specificity of the centromere in the chromosomes of *Crepis* and other genera was noted by Trankowsky (127). Attachment of trabants (satellites) to nucleoli in prophase of mitosis was reported in *Crepis* by Navashin (*cf.* 18) and confirmed by Matsuura (72) and others. The hypothesis of Heitz (49) that nucleoli arise only on certain chromosomes was based partly on evidence from *C. capillaris.* Heitz's (49) observation of a constant relation between distal as well as proximal satellites and nucleoli was made in *C. pulchra;* constancy in position of the nucleoli with reference to the chromosomes was found in *Crepis* species; and a constant relation between number of satellites and number of nucleoli was observed in *C. capillaris, C. sibirica* and *C. pulchra.* Geitler (40) confirmed the association of nucleoli with satellites in a tetraploid root of *C. capillaris.* Heterochromatic regions were observed by Kostoff (58) in the chromosomes of root tips of *Crepis* and *Triticum* which were stained with haematoxylin and differentiated more than usual. These, it was suggested, may correspond to the genetically "inert" regions in Drosophila chromosomes.

Induced Structural Variations

By the use of x-rays. Experimental alteration of the chromosomes in *Crepis* has received considerable attention, especially among Russian investigators. Navashin (75, 79) initiated experimentation with the effects of x-rays on *Crepis,* using *C. tectorum* and treating moist seeds. On the basis of extensive observations, the following categories of chromosomal alterations were reported: *a) quantations, i.e.,* addition or subtraction of entire haploid sets or entire single chromosomes; *b) dislocations, i.e.,* rearrangements of chromosome material such as attachment, duplication, transloca-

tion, fragmentation, *etc.; c) transformations, i.e.,* alteration of the morphological characters of the chromosomes, invariably connected with a change in the total mass of the nucleus, although often affecting different chromosomes independently; *d) novations, i.e.,* a presumed phenomenon of formation of chromosomes *de novo.* This last category should probably be merged with translocations. Navashin (79) also stated that transformations are believed to have occurred in the course of the evolution of the *Crepis* species which in most cases differ markedly in the sizes and shapes of their individual chromosomes Then followed a series of papers by Levitsky and several associates (68, 69, 70, 71), all concerned with *C. capillaris,* in which instances of chromosome alteration were reported, but in which it was assumed that all of the translocations observed were non-reciprocal in nature.   In 1935, however, Levitsky (66) concluded that the translocations induced in *C. capillaris* by x-ray treatment are mostly if not all reciprocal.   He inferred that localization of breakages followed by translocations is conditioned by some internal peculiarity of the chromosome, perhaps purely mechanical, such as a lesser contraction of the chromonema in those regions.   He concluded that breakages leading to reciprocal translocations occur shortly before the metaphase stage. These conclusions were drawn from the analysis of somatic plates in which the "shortening curves," representing changes in the longer arm of all 3 chromosomes, are compared.   In 1940 Korjukaev (57) corroborated Levitsky's conclusion that the translocations induced by x-rays in *C. capillaris* are reciprocal.   This was based on a careful analysis of 2 cases involving translocations between the *A* and the *C,* and between the *A* and the *D* chromosomes. In 1937 Levitsky (67) reported that the chromosomes in *C. capillaris* are very stable, but that among 295 plants from x-rayed seeds there were 25 deviations in chromosome morphology.   These were confined to 11 out of 28 families, and 2 of these families had 11 of the 25 cases.   Hence, he concludes, there are genetic differences conditioning structural instability and these appear to be an important cause of karyotype evolution.   In 1940 Levitsky (131) published supplementary data on x-rayed *C. capillaris.*   Out of 491 seedlings, 84 or 17.1% were abnormal in karyotype; and 74 or 15% showed structural chromosome changes, including reciprocal translocations, inversions and duplications.   Some of these aberrant

plants had lowered fertility which was usually more marked when the plant was heterozygous for the chromosome abnormality for cytological reasons. But some abnormal homozygotes also had low fertility, due perhaps "to a lethal expression of the 'position effect.' "

By other agents. Other external agents which have been applied for the purpose of inducing chromosomal changes in *Crepis* are acenaphthene and centrifugal force. In the cells of *C. capillaris* root tips, after treatment with acenaphthene, Navashin (89) found haploid, tetraploid and octoploid cells. As a result of centrifuging germinated seeds of *C. capillaris,* Kostoff (60) found simple and complicated reorganization of the chromosomes. Some chromosomes with dislocated segments were formed. All the plants were chimerical.

By the aging of seeds. The aging of seeds as a cause of chromosomal mutations was reported by Navashin (84) in 1933. Seeds of *C. tectorum* show only about 2–3% germination when kept 6–7 years. Chromosome translocations, and possibly one inversion, were found in 81% of the plants. The opinion was expressed that such mutations in nature must be infrequent, since seeds are not preserved long past the germination period, except under artificial conditions. The same year Navashin (86) announced that the process of mutation in resting seeds was accelerated by increased temperature. Shkvarnikov and Navashin (110) reported that fresh seeds of *C. tectorum* after exposure to 54°–55° C. for 20, 40 and 44 days, showed progressively less germination, longer germination periods, and chromosome abnormalities, just as they did when exposed to increasing doses of x-rays. Out of 106 roots of surviving plants (from 20 days exposure), 14 showed chromosome translocations.

Navashin and Gerassimova (90, 91) confirmed the earlier report that chromosomal dislocations occur in the meristematic cells of the resting embryos of seeds. These mutations occur in the resting nuclei and are manifested in the first cell divisions at onset of germination. In the earliest stages of development, a great variety of chromosomal aberrations may be observed in individual cells, the majority leading to the elimination of the cells containing them. In seedlings capable of further development, only a few mutations are preserved, and a chimerical plant, usually consisting of 2 com-

ponents, mutant and normal, arises. The viability of mutant individuals which survive the critical period soon after germination is often normal, though abnormalities are very common and their fertility is often much reduced. When offspring are obtained, the transformed chromosomes are transmitted. The great majority of viable chromosomal mutations are translocations; there is relatively little evidence that inversions have occurred. Each of the chromosomes is involved in translocation with nearly the same frequency. Shorter chromosomes more often act as "recipients" than "donors" and longer chromosomes conversely, meaning that longer chromosomes lose more, and shorter ones less chromatin. The same is true for shorter and longer arms of a chromosome. The evidence does not prove that all the translocations are reciprocal, but this is very probable. The marked frequency with which these alterations occur shows that the homologization of chromosomes of different species solely on the basis of their morphological characters is no longer tenable and may often lead to great confusion. Certain of these data furnish good support for the "dislocation hypothesis" of evolution of basic chromosome numbers. Furthermore, translocations and inversions should play an important rôle in evolution as factors causing physiological isolation (cf. 34, 42). These mutations are also of importance in plant breeding and seed production. It seems probable that gene mutations also occur as a result of the aging of seeds. Mutations, like any other physiological process, must be conditioned by the environment. Hence, a study of the correlation between frequency of mutation and various natural factors, particularly temperature and moisture, is highly desirable.

More recently, Shkvarnikov (109) has reported that temperature, humidity and other factors cause mutations in stored seeds through the physical and chemical processes taking place in the seeds; and similarly in the case of mature pollen. Mutations of various sorts occurred—lethal and viable, large and small, chromosomal and "point," positive and negative. Thus, in nature such variations must occur with high frequency when favorable conditions exist.

Lastly, Navashin, Gerassimova and Belajeva (93) made a careful study of C. capillaris and C. tectorum plants grown from seeds stored for 2, 3, 4 and 5 years in a basement where temperature and

humidity fluctuations were recorded. The mean temperature was 18.7° C. with a maximum seasonal range of 16.4°–20.5°. The humidity was much more variable, ranging from 40–50% in winter to a maximum of 80% in midsummer. A striking difference between the two species occurred in the effects of storage on germinating power and viability of the seedlings. At the end of 2.5 years all the seeds of *C. tectorum* had nearly lost the ability to germinate and the viability of the few seedlings obtained was almost nil, whereas the seeds of *capillaris* showed much greater resistance, as had been observed in earlier work. This distinction between the two species, it is pointed out, is an important adaptation. *C. capillaris* is a typical annual, and its achenes normally germinate in the spring after a period of 250–270 days of dormancy, whereas *C. tectorum* is a "winter plant," *i.e.,* its seeds germinate soon after maturation and the vegetative rosettes live through the winter, the plants flowering the following spring or early summer. (It may be noted that this physiological peculiarity of *tectorum,* combined with evidence from comparative morphology of the plants and the chromosomes as well as geographic distribution, has been of value to the writer in determining the interspecific relationships of *C. tectorum.*)

Since *C. capillaris* proved to be a favorable species, thoroughgoing study was made of the percentage of occurrence of structural chromosomal mutations and percentage of mortality among embryos and seedlings correlated with increasing storage periods. From 7,761 seeds stored, 1,220 seedlings were obtained, and 967 survived, of which 737 were examined cytologically. The important fact established was the marked difference between rate of loss of germinating ability of the seeds and rate of occurence of chromosomal mutations in the embryos. The percentage of total mortality among embryos and seedlings increased uniformly at the end of 3, 4, 5 and 6 years; whereas the percentage of chromosomal mutations was proportional to the time lapse only for the first two years of the period of observation and then showed a very rapid increase. That is, "after a lapse of 1000 days the proportion of mutations begins to grow rapidly. Thus, a further 1.2-fold increase in the period of rest is connected with a nearly 5-fold period in percentage of mutations; a 1.5-fold period leads to a nearly 9 times greater proportion of mutations, *etc.* There is thus no doubt left behind

that we actually are dealing here with a biological process rather than with a mere accumulation of direct effect of external influence." The fact that the proportion of mutations was greater in these seeds than in seeds stored under ordinary room conditions led to the inference that increased humidity was the determining factor, which suggested that seeds stored naturally in deep crevices, caverns, or any cool place where condensation of moisture takes place during the summer should contain a greater proportion of mutations than seeds stored under conditions where the humidity of the air is low.

Spontaneous variations. Spontaneous structural chromosomal alterations have been reported by Navashin (80, 82, 85) and Swezy (122, 123) in various species of *Crepis.* The significance of such spontaneous mutations for the evolution of *Crepis* was emphasized by Navashin (80). "It is obvious that any heterozygous dislocation (translocation) would ultimately result in gain or loss of chromatin material in succeeding generations owing to segregation of chromosomes. And, if not incompatible with life, these may result in variations of evolutionary significance. For there can be hardly any doubt that the evolution of *Crepis* species was primarily based upon changes in the quantity of material contained in the individual chromosomes."

Structural Changes as an Evolutionary Process

Structural changes in the chromosomes, leading to chromosomal transformation, alteration of karyotypes and changes in chromosome number, have long been recognized (Hollingshead and Babcock, 53) as of fundamental importance in the evolution of *Crepis.* In the light of accumulated evidence, the conclusion that this process has been of primary importance in *Crepis,* and probably in several related genera, appears to be fully warranted. The hypothesis of Delaunay (33) that *reduction in absolute size of the chromosomes often accompanies evolutionary advancement* is in general agreement with the evidence on phylogeny, as based on comparative morphology of the plants and on chromosome size, not only in *Crepis,* but also in *Lactuca, Prenanthes* and *Ixeris* (14, 20); although there are certain exceptions to the general rule. The hypothesis of Levitsky (65) that *primitive species have more numerous chromosomes with median constrictions,* with the chromosomes

of a set nearly equal in size, and that *along with advancing evolu-tion subterminal constrictions are developed* and the chromosomes of a set become fewer in number and more unequal in size, is sup-ported by the evidence from *Crepis* and from related genera (20).

Navashin's dislocation hypothesis. In view of her evidence on partial metaphase pairing in hybrids between *Crepis* species with different chromosome numbers and different karyotypes, Avery (1) inferred that the chromosomes of the different species con-tained some genically homologous segments. She suggested a scheme of karyotype transformation, involving reduction in number from 5 to 4, based on several types of structural change. Such evi-dence, together with the apearance of new types of chromosomes in the progenies of two triploid hybrids, *C. capillaris × C. aspera* (*cf.* 18) and *C. capillaris × C. tectorum* (46), led Navashin (83) to propose his "dislocation hypothesis." Abandoning any simpler fragmentation-fusion hypothesis, he recognized the following facts: *a*) chromosome number is conditioned by the number of centro-meres which can not be formed *de novo; b*) segments of chromo-somes may be lost or transferred to other chromosomes; this "dis-location" process occurs in both somatic and germ cells; *c*) the normal effect of a given portion of chromatin does not depend on the particular place it occupies in the chromosome system; and regular pairing in meiosis does not depend on the similarity of whole chromosomes but on similarity of elements occupying corre-sponding levels in the conjugants. Hence, *the only conceivable way of changing the basic chromosome number is addition or loss of one or more centromeres combined with appropriate dislocation. Change in the plus direction* could be accomplished by, first, addi-tion of an extra chromosome by one of several known ways; sec-ond, replacement of the chromatin on each side of the centromere in this extra chromosome, or one of its 2 homologues, with material derived from one or more non-homologous chromosomes; third, origin, through sexual reproduction and segregation, of an indi-vidual homozygous for the new extra chromosome. *Change in the minus direction* could result from, first, loss of material from one or more chromosomes through translocation to other chromosomes; second, elimination of the remaining centromeres and their normal partners through loss or segregation; third, origin of an individual homozygous for the reduced chromosome number. It will be noted

that non-reciprocal translocations were assumed, but that later evidence proved reciprocal translocations to be the rule in *Crepis*. Also, that trisomics are always less viable and fertile than normal diploid plants, especially in species with such low numbers as the Old World diploid species. Furthermore, the "dislocation hypothesis" was admittedly inadequate, since it dealt only with chromosome number as a specific charactertistic. However, it will also explain changes in symmetry or individual chromosomes and in the relative size of different members of a set. But it leaves unexplained the evolutionary transformations in absolute size and bulk of the chromosomes.

Evidence has continued to accumulate, however, showing that structural changes have occurred during the evolution of *Crepis* species. The most convincing of such evidence has come from the meiotic irregularities found in hybrids between natural species (74, 44; Sherman, unpublished; Tobgy, unpublished) which are discussed below in connection with interspecific hybridization as a factor in evolution. But it should be recognized here that such irregularities are caused by differences in location of homologous segments in the chromosomes *as they existed in the two species before they were crossed*. How did these structural differences in the parental species arise? An answer to this question can now be found in the results of experiments on induced structural changes and on the occurrence of structural changes in the embryos of seeds stored under conditions of high humidity and high temperature.

Gerassimova's achievement. Karyotypically distinct new forms of *Crepis tectorum,* produced by treating pollen and moist seeds with x-rays, have been investigated by Gerassimova (45). The main steps in the process of producing these new forms, necessarily omitting many details, are as follows. Two different reciprocal translocations were found among the progeny from x-rayed material. One involved the A and D chromosomes, and the original plant was heterozygous, but among its progeny an individual homozygous for the same structural change was found. The other original mutant was already homozygous for a reciprocal translocation between the B and C chromosomes. Both of these homozygous translocants were morphologically identical with normal *tectorum* and just as self- and cross-fertile. By crossing these two homozygous strains, F_1 hybrids were obtained in which each of the 4 chromosome pairs

differed structurally; but the plants differed from normal *tectorum* only in fertility. They produced 31% of good seeds as compared with 80% in normal controls. The progeny obtained by selfing these hybrids contained some plants with normal karyotypes, and the others had all the possible combinations of heterozygous and homozygous translocations, including one which was homozygous for translocations in all 4 chromosome pairs and which was called *C. nova I*. In the next generation, a plant was found that was homozygous for strikingly different translocations in all 4 pairs; it was called *C. nova II*. Both of these karyotypically new strains were morphologically identical with normal *tectorum* and equally viable and fertile; also, just as uniform except for an occasional trisomic plant in *C. nova I* because of the small size of 1 of the 4 pairs. But, when *C. nova I* was crossed with normal *tecorum,* the F_1 hybrids were only 30% fertile when self-pollinated and slightly more when open-pollinated. A careful study of meiosis in these hybrids revealed chromosome behavior in full accord with expectations, including formation of quadrivalents involving normal chromosomes and their translocated mates; and it is assumed that all gametes, except those resulting from alternating distribution of the chromosomes to the poles, are inviable. It is also assumed that part of the sterility may be due to other causes.

Whatever the precise nature of the sterility may be, there has been created here a highly efficient genetic-physiologic mechanism, causing isolation between two constant forms of the same species (*cf.* 34). It is also very probable that crossing over between homologous segments will produce further structural changes resulting in additional sterility. "It becomes inevitable, therefore, that the progeny of the hybrids in question contains a very limited proportion of individuals with intermediate characters, but consists of the two parental types and of individuals which repeat the original hybrid. In other words, there exists a situation characteristic of a hybrid between two genuine species." Although *C. nova I* and *C. nova II* are still morphologically indistinguishable from the original *C. tectorum,* "accumulation of mutational changes should undoubtedly lead in future to such distinction."

Although not suggested by Gerassimova, it should be mentioned that most or perhaps all of these new mutations, leading to morphological and physiological differentiation, would probably be in

the nature of gene or point mutations. And it should be emphasized that this expression "gene or point mutation," as used throughout this review, is intended to convey a very general notion of any sort of change in the material at a given locus of a chromosome. It should also be recognized that the initial sterility which is set up by the gross structural changes (translocations) in the new *tectorum* forms, *C. nova I* and *C. nova II,* is only partially characteristic of interspecific sterility in general. That sterility of a more complex nature would be built up, however, along with morphological diversity by ensuing gene mutations is clearly indicated by the evidence on partial intersterility between species which differ only in Mendelian variations.

This outstanding achievement in experimental evolution is the second[2] adequate response to Bateson's (23) famous demand: *"The production of an indubitably sterile hybrid from completely fertile parents which have arisen under critical observation from a single common origin is the event for which we wait."* All of the conditions stipulated here are fully met, and the "evolutionary faith" which Bateson so staunchly maintained has now become firmly grounded by this "acceptable account of the origin of 'species.'"

It has now been fully demonstrated that chromosome transformations similar to those experimentally induced by Miss Gerassimova (45), are produced in considerable numbers in normal dormant seeds when stored under conditions of high humidity or high temperature. They also occur "spontaneously," though more rarely, in seeds stored under ordinary conditions. When one considers the almost infinite variety of conditions under which seeds in nature may await a suitable opportunity for germination, it seems probable that here is an important natural source of this category of genetic evolutionary processes in plants, viz., structural changes in the chromosomes.

CYTOGENETICS OF DIPLOID SPECIES AND HYBRIDS

Intraspecific Differences

Accumulation of data on the inheritance of character differences in *Crepis* species has not been a major part of these investigations.

[2] In 1934 Dubinin (Jour. Biol. Moscou 3: 719–736) reported a comparable achievement, using *Drosophila melanogaster.* The partial sterility of aneuploid forms, such as the *Datura* trisomics, is less significant for evolution in general.

The promise of results of significance to cytotaxonomy which might be obtained immediately from hybrids between genetically unanalyzed species has led the genetical studies along these lines. Some data on the inheritance of character differences have been reported for the following species, some of the data having been obtained from interspecific hybrids: *Crepis alpina* (24), *aurea* (18), *bursifolia* (18), *canariensis* (55), *capillaris* (18, 50), *Dioscoridis* (111), *divaricata* (55), *eritreënsis* (15), *foetida* (15, 18, 98), *leontodontoides* (18), *Marschallii* (18), *neglecta* (18), *nicaeënsis* (16), *parviflora* (18), *rubra* (18, 98), *setosa* (16), *syriaca* (24), *tectorum* (18, 43, 50, 52), *Thomsonii* (15), *vesicaria* (55).

Interspecific Hybridization

Interspecific hybridization has been carried out on an extensive scale. The compatibilities of 35 species, between which hybrids were obtained before 1930, as indicated by seed production under open-pollination, were reported by Babcock and Navashin (18). Since then many more hybrids have been obtained. Between the years 1920 and 1939 data were obtained on about 200 interspecific hybridizations involving 55 different species. The data on crossability, viability and fertility have not yet been published. In general, it can be stated that, as would be expected, hybrids between species which are less closely related, as judged from morphology, tend to be weak and sterile or, if vigorous, to be sterile or of very low fertility; while hybrids between more closely related species tend to be vigorous and more or less fertile.

Evidence of simple and complex Mendelian segregation has been observed in the F_2 and backcross progenies of numerous interspecific hybrids in *Crepis*. The most extensive data have been reported on 2 groups of very closely related species. These are among the least differentiated genetically of all the natural units which are recognized as species by the writer. Babcock and Cave (15), working with *C. foetida, C. eritreënsis* and *C. Thomsonii,* found segregation in F_2 families giving monohybrid, dihybrid and trihybrid ratios for certain structural and qualitative differences, whereas such quantitative differences as leaf shape and stem height, also self-compatibility *vs.* self-incompatibility, displayed variable proportions in F_2, suggesting that these differences are probably conditioned by several or numerous genetic factors. Results of

further study on this group by Hughes (unpublished) are in general agreement with the evidence just mentioned concerning factors affecting degree of compatibility. Jenkins (55) worked with *C. divaricata, C. Noronhaea, C. canariensis* and *C. vesicaria.* He found that most of the parental differences under investigation exhibited the blending type of inheritance in F_2 which indicates that these species differ in a large number of genetic factors and that most, if not all, of the specific differences were influenced directly by many genes. A few characters, those conditioned by a single gene differential, showed dominance in F_2. In other words, most of the specific differences are gene determined but do not conform to a simple Mendelian scheme. The internal mechanisms isolating the species was found to be incomplete, and varying degrees of congruity between them were indicated by the comparative fertility of the hybrids. All the evidence indicates that the isolating mechanisms that have been built up between these species are due to gene incompatibilities, which undoubtedly have arisen over a long period of time, and there is no indication whatever of any chromosomal rearrangements.

One of the earliest products of interspecific hybridization in the genus was *Crepis artificialis* (18, 125, 28) which arose from the cross, *C. setosa,* $n = 4, \times C. biennis, n = 20$, as a result of the peculiar distribution of the parental chromosomes to the F_1 gametes. As a result of autosyndesis among the 20 *biennis* chromosomes and random distribution of the 4 *setosa* chromosomes, a segregant was obtained having 24 chromosomes, consisting of 20 *biennis* and 2 pairs of *setosa* chromosomes. The first few progenies of *C. artificialis* were fairly uniform, and it was planned to test the "new species" under natural conditions. But later, considerable variation was observed among progenies from selfed plants, and, as a result of testing selected individuals, Jenkins (unpublished) has obtained strains with various diploid numbers ranging from 20 to 36. Some of the strains thus established appear to be fairly uniform morphologically. That *C. biennis,* $2n = 40$, is an octoploid with the base number, $x = 5$, was indicated by the fact that a backcross of *C. artificialis,* $n = 12$, to *C. setosa,* $n = 4$, formed at first meiotic metaphase 7 pairs and 2 single chromosomes, which was interpreted by Collins, Hollingshead and Avery (*cf.* 18) as 5 *biennis* pairs + 2 *setosa* pairs + 2 *setosa* singles. Corroborative evi-

dence was found (22) from a study of the karyotype of *C. biennis* in which it was possible to demonstrate a basic idiogram of 5 distinct chromosomes.

Amphidiploid hybrids have arisen spontaneously from three different interspecific crosses: *Crepis capillaris × C. Dioscoridis* (18) ; *C. capillaris × C. tectorum* (49) ; and *C. rubra × C. foetida* (98, 99). The first two were completely or almost completely sterile. The third produced some seed but the most fertile second-generation plants obtained were not over 10% fertile, and it was concluded that stable races are not likely to be derived from this amphidiploid. But that amphidiploidy has been of importance in the origin of certain *Crepis* species, is shown below.

An intergeneric hybrid involving *Crepis capillaris, n = 3*, and *Taraxacum platycarpum, n = 8*, was reported by Sinoto and Ono (112). Most of the seedlings obtained had either 6 or 16 chromosomes but 1 which showed intermediate characters was very delicate, died before it could be examined cytologically, and may have been a hybrid. The purported intergeneric hybrid of *Paraixeris denticulata × Crepidiastrum lanceolatum* var. *latifolium* (132, 96, 94) is changed to the category of interspecific hybrid, since the reduction of *Paraixeris* to *Ixeris*, by Stebbins (115). *Crepidiastrum lanceolatum* was at one time known as *Crepis integra* Miq.

The Rôle of Gene or Point Mutations in Evolution

The view, often advanced in the past, that gene mutations produce only abnormalities and other deleterious effects and hence can not play any rôle in evolution is quite untenable, as was pointed out by Sansome and Philp (106). In *Crepis,* as well as many other organisms, allelomorphic characters are known to have no effect whatever on the viability, vigor or fertility of the individual. Numerous morphological differences of this sort have been reported in *Crepis* and two of these deserve to be especially mentioned.

Receptacular paleae. Crepis, like other genera in the Crepidinae, is generally characterized by absence of paleae (small bracts) on the receptacle, although a few species of *Crepis* and one monotypic American genus have them in reduced form. Among the F_1 hybrids from a cross between different strains of *C. capillaris,* both lacking paleae, Collins (*cf.* 18) obtained plants, otherwise normal, with a bract subtending each floret. This reversionary type of re-

ceptacle behaved as a simple Mendelian recessive in all further crosses. It may, therefore, be concluded that an important step in the evolution of the Crepidinae was the dominant gene mutation for absence of paleae. In *Crepis foetida* (15) paleae are normally present in subsp. *commutata,* whereas in subsp. *vulgaris* they are always absent and in subsp. *rhoeadifolia* they are usually absent. In this species the inheritance of presence and absence of paleae follows a different pattern, presence being dominant to absence. The data from numerous crosses establish the existence of duplicate dominant genes for paleae and of a dominant inhibitor, epistatic to one of these genes and hypostatic to the other. Thus at least three gene mutations are involved in the evolution of the subspecies with naked receptacles in this species.

Interspecific lethals. An interspecific lethal factor was discovered by Hollingshead (52) in *Crepis tectorum.* It was found that some strains of this species, when crossed with *C. capillaris,* would produce only plants which died in the cotyledon stage, whereas others would give all viable offspring, and still others about half-and-half viable and inviable. Further tests proved that this factor behaved as a simple Mendelian recessive. Since normal meiosis in *C. tectorum* is always regular it may be assumed that this factor is not a structural difference in the chromosomes in the different strains of *tectorum* but simply a gene, using that term in the sense of a qualitative difference of some sort at a given point in a given chromosome. Hollingshead (52) also showed that, in crosses between *C. tectorum* and *C. bursifolia* and between *C. tectorum* and *C. leontodontoides,* both viable and inviable progeny occurred in the proportions expected if the *tectorum* lethal were effective. But her data on *C. tectorum* × *C. vesicaria* ssp. *taraxacifolia* and on *C. tectorum* × *C. setosa* indicate that the lethal is ineffective in these hybrids. Certain data of Haney (unpublished) on other interspecific crosses, not involving *C. tectorum,* suggest that similar lethals may exist in other species of *Crepis* but these have not been subjected to further investigation. It should be noted that some of the *tectorum* strains used by Hollingshead were of wild origin, in widely separated localities, and the others were from various botanical gardens in widely different regions. Evidently this gene mutation occurred either long ago or more than once in the history of this species. Now that the closest relatives of *C. tectorum* are be-

lieved to be *C. Bungei* and *C. ircutensis*[3] it would be of interest to investigate the behavior of hybrids between these species and *tectorum*. If the lethal gene proved to be effective in these crosses, the evidence of its importance in the evolution of *tectorum* through isolation would be considerably enhanced.

Intraspecific differentiation. Although such individual gene mutations as those causing naked receptacles and interspecific lethals are doubtless of importance in the evolution of *Crepis,* yet it is the great mass of gene mutations, affecting all parts of the plant, which provides most of the material for intraspecific differentiation. And the evidence from *Crepis* certainly indicates that, given some kind of isolation—geographic, ecological or physiological—intraspecific differentiation leads eventually to the origin of new species. Many species of *Crepis* are polymorphic, consisting of more or less well-defined geographical races or subspecies, with identical karyotypes, which have been shown to differ from one another only with respect to certain genes (18, 15). Such species are in process of differentiation as a result of gene mutation and selection under varying environmental conditions. Excellent examples are *C. foetida* and *C. vesicaria,* both of which are composed of several morphologically distinct but interfertile subspecies, each with a wide distribution, but overlapping sufficiently so that swarms of intergrading hybrid forms occur. Hence, under existing conditions, each of these 2 species is actually a rassenkreis. Yet it is easy to imagine what would happen if some sort of barrier like intersterility were to separate one of the subspecies from all the rest. Its status would change to that of species in course of time, if not rapidly. This in effect is what has happened in the case of 2 groups of very closely related *Crepis* species which have been rather thoroughly investigated, only here the isolating barrier is geographic instead of physiological.

a) Crepis foetida and two close relatives. In addition to *C. foetida,* Babcock and Cave (15) studied *C. eritreënsis* and *C. Thomsonii,* the last being restricted to northern India and the other to Eritrea, while the polymorphic *C. foetida* extends throughout southern Europe, Asia Minor and eastward into Persia. The 3 entities have closely similar karyotypes and in F_1 hybrids between them meiotic regularity is practically perfect, indicating no structural differences. F_1 fertility varies from very high in some crosses

[3] *Crepis ircutensis* Babcock, Univ. Calif. Pub. Bot. 19 : 401, 1941.

to very low in others. Morphologically the plants are generally similar, but they are distinguished by marked differences in their flowers, fruits and involucres as well as other parts; and sufficient genetic evidence exists to show that they differ from one another in numerous genes. As stated above, the F_2 variation from crosses involving differences in quantitative characters and self- *vs.* cross-compatibility indicated that they are conditioned by several or many genes. More extensive crossing experiments would probably reveal many other genic differences between them. However, if the distributional areas of *eritreënsis* and *Thomsonii* overlapped that of *foetida,* the first two would probably intergrade with *foetida* through crossing. They would then have to be considered as subspecies of *foetida.* For this reason, and because they differ from *foetida* only in Mendelian factors, Goldschmidt (48, pp. 99–101, 159) claims that they, too, are subspecies. Having thus disposed of them, he cites this case as an excellent example of interspecific differentiation or "microevolution," in support of his contention that Mendelian differences are due to micromutations which have nothing to do with the origin of true species. Real species, he claims, arise only through macromutations, or systemic alterations which insure physiological isolation.

Meanwhile, it must be admitted that *C. eritreënsis* and *C. Thomsonii* have an ideal opportunity to show future biologists whether or not they are capable of becoming so widely differentiated from *C. foetida* that no biologist would think of considering them anything but true species. To be sure, the present extent of the *eritreënsis* population is not known and it may be small, which would be a limiting factor. But *Thomsonii* covers a wide area and is already rather variable in response to environmental differences. The present writer is content to leave it for the future to decide the fate of these two entities. That they are actually incipient species, seems to him strongly indicated by the following facts: *a*) they differ from *foetida* in many, not few, genes (15); *b*) F_2 data from crosses show that these 2 species differ more from *foetida* in respect to self- and cross-compatibility than the *foetida* strains differ among themselves (15. p. 140); *c*) they both differ genetically from *foetida* as to color of the ligules and the presence of red ligule-teeth (15, pp. 147, 149); *d*) *C. eritreënsis* has monomorphic achenes, while *Thomsonii* and *foetida* have dimorphic achenes (15, p. 149);

e) both species differ from *C. foetida* and from each other in other morphological characters and both have a shorter life-cycle than the most precocious form of *C. foetida;* *f*) both species show more morphological and physiological resemblance to *C. foetida vulgaris* than to the other 2 subspecies of *foetida,* but the former subspecies is farther removed from them geographically. This indicates a phylogenetic connection fairly remote in time. During an ensuing epoch, barring a catastrophe and assuming continuation of the present rate of gene mutation, these species may become much more distinct from *foetida* than they are now. Meanwhile, a structural change leading to intersterility could arise at any time. These speculations regarding the future, however, are purely gratuitous and not essential in reaching a decision.

Recognition of *C. eritreënsis* and *C. Thomsonii* as species is in full agreement with the following principles, advocated by Huxley (54, p. 22) : "As Turrill (128) has emphasized, the fact that groups may or might show fertile intercrossing when artificially or in other ways secondarily brought together does not disprove their right to be styled species. It is the actual facts of nature, not its every potentiality, with which the systematist has to deal. The fact of their separate existence *qua* self-perpetuating interbreeding groups, together with *either* a reduction or absence of fertility in intercrossing, *or* a certain empirically evaluated degree of morphological or physiological characters, should be taken as the basis of decision." It is the opinion of the reviewer that the two entities in question have sufficient morphological and physiological distinctness to justify their recognition as species and that invoking their present interfertility as the sole criterion against such recognition is unwarranted.

b) *Four island endemics.* The other group of closely related species was investigated by Jenkins (55). The group consists of 3 endemic species, isolated on the Madeira and Canary Islands, *C. canariensis, C. Noronhaea* and *C. divaricata,* together with 2 subspecies of *C. vesicaria viz., andryaloides* and *taraxacifolia,* all with $n = 4$. The latter subspecies must have been introduced into Madeira by early Portuguese colonists. It is fully naturalized and has spread in the vicinity of Funchal and along a trail across the island to the north side, where it came in contact with one of the endemics, *andryaloides.* There the two have intercrossed and produced a

swarm of fertile hybrids. Hence *andryaloides* should be tre
a subspecies of *C. vesicaria*. In general, the facts about the
entities, as regards morphological similarities and differences, ka
yotype similarity and meiotic regularity in parents and hybrids, and
the genetics of quantitative and qualitative differences, are prac-
tically identical with those concerning the 3 species considered
above. The 3 island endemic species now under consideration are
effectively isolated; and *andryaloides* was also until *taraxacifolia*
became naturalized and invaded its area. Although *andryaloides*
and *divaricata* both occur only on Madeira itself, the former is an
upland, and the latter a lowland form which is now restricted to the
easternmost promontory just above sea-level.

In his experimental F_1 hybrids between the 4 species, Jenkins
(55) found that the average fertility, as indicated by percentage of
seed setting in the open, was 25–50%. The least fertile hybrids
had only 1–2%, and the most fertile, 50–75%, as compared with
nearly 100% in all of the parents. Thus, interfertility between all
4 species has been definitely though not completely reduced, evi-
dently as a result of gene mutations. According to Jenkins (55, p.
382): "The cytological evidence strongly indicates that the 5 enti-
ties have a similar arrangement of genes in the various chromosome
types. In other words, there have been no large duplications, trans-
locations, or other rearrangements that in any way interfere with
normal meiosis." From this it may be assumed that most if not all
of the genetic variations between the 5 entities are the result of gene
or point mutations. Furthermore, Jenkins (55, p. 394) states:

"Among the 5 entities there were a great many morphological
differences which affected all parts of the plant. In the hybrids by
far the greater number of these differences appeared to be the result
of the presence of a large number of multiple genes. . . . The in-
ternal isolating mechanism between them was found to be incom-
plete, although varying degrees of congruity between them were
indicated by the comparative degree of fertility of the hybrids. For
practical taxonomic purposes, the fact of geographic or ecologic
isolation warrants the recognition of *C. divaricata, C. Noronhaea,
C. canariensis,* and *C. vesicaria* as species; whereas *andryaloides*
and *taraxacifolia* must be considered as subspecies of *vesicaria* be-
cause they are hybridizing in nature and losing their morphological
distinctness."

ertheless, Goldschmidt (48, p. 159) finds here further evi-
.e to support his hypothesis that Mendelian mutations can not
.iction in the origin of species, simply because he believes these 5
entities must all be considered to be subspecies. Let us again con-
sider the facts. In this second group of closely related species we
find not only good morphological distinction and differences in
many genes, but as a result of gene mutations there exists a partially
developed internal isolating mechanism. This type of isolating
mechanism has been used by Clausen, Keck and Hiesey (27) as a
criterion for distinguishing ecospecies which usually correspond to
taxonomic species.

After all, the important question is not concerned with the classi-
fication of certain forms as specific or subspecific—that remains to
some extent a matter of individual judgment. The question really
at issue is whether or not gene or point mutations are operating in
nature so as to create intraspecific, physiologic isolation. The evi-
dence from Jenkins' island endemics certainly indicates that they
are so operating. In this connection it is also worth noting, as has
already been recognized by Sansome and Philp (106, p. 321), that
in two widely distributed polymorphic species of *Crepis,* in which
well marked geographic races (subspecies) have not been recog-
nized, similar evidence on intraspecific sterility has been reported.
In *C. capillaris* a low diffuse form, found in the Pyrenees, when
crossed with a robust form from northern Europe, gave an F_1
which was intermediate in size and habit and was not over 50%
fertile. In *C. tectorum* a cross between a Scandinavian dwarf
form, known as var. *pygmaea,* and a tall form from Russia also gave
an F_1 intermediate in size and habit and with 50–60% estimated
fertility. Meiosis was not studied in these hybrids, so that the evi-
dence is not complete. But, with more extensive research on intra-
specific hybrids in *Crepis,* there can be little doubt that many more
cases of partial sterility between subspecific entities, having no de-
tectable structural differences in their chromosomes, would come
to light.

From the evidence reviewed above it is very clear that in the
genus *Crepis* gene mutations certainly do comprise an evolution-
ary process of major significance. In addition to their function in
building up intra- and interspecific sterility, because of the fact that
such mutations are omnipresent, they may at any time operate to

supplement and extend the other genetic processes concerned in the development of new species.

Meiosis in Crepis Species

The importance of meiotic behavior of the chromosomes in species and interspecific hybrids in determining species relationships was discussed by Darlington (30) and 6 categories of species were mentioned, *viz.,* self-fertile diploids, cross-fertilized diploids, sex-fertile polyploids, mixtures of the first three, complex heterozygotes, and clonal species which reproduce by apomixis. The diploid species of *Crepis* in general are highly regular in their meiotic behavior. J. Clausen (*cf.* 18) observed perfect pairing at first metaphase in *C. aspera* and *C. bursifolia.* Complete or nearly complete regularity was reported in *C. tectorum* by Hollingshead (50) ; in *C. foetida* and *C. eritreënsis* by Babcock and Cave (15) ; and in *C. divaricata, C. Noronhaea, C. canariensis* and *C. vesicaria* by Jenkins (55).

In the "X-strain" of *C. capillaris,* however, Hollingshead (50) found univalent chromosomes at metaphase in from 12 to 44% of the pollen mother cells in different plants of this strain growing under the same favorable conditions. It has been discovered by Richardson (103) that, in progeny from a diploid branch of a haploid individual of this same strain of *capillaris,* the occurrence of univalents at first metaphase is caused by failure of chiasma formation between homologous chromosomes *which are found to be regularly associated at pachytene*. She reports that such univalent chromosomes frequently lie in juxtaposition at diakinesis, presumably a result of their earlier association. Whatever may have caused failure in chiasma formation, it is clear that marked irregularities in metaphase pairing may occur in morphologically similar individuals of the same species. Such marked variability between individuals, however, is not generally characteristic of diploid species. Hollingshead (51) studied metaphase pairing in the haploid plant and its diploid branch which furnished the X-strain material on which Miss Richardson worked. Meiotic behavior in pollen mother cells of the haploid was very irregular and variable, with random segregation of univalents at the first division, and rarely the division of all univalents followed by separation of the daughter halves to different poles. In diploid tissue on the haploid plant 3

bivalents were frequently observed, but non-conjunction of 1 or more chromosome pairs was frequently observed just as in plants of the original X-strain.

From a study of the chromosomes present in the pollen grains of a triploid *C. capillaris* plant, Chuksanova (25) determined that all 8 possible combinations of the 3 pairs of chromosomes actually occurred at metaphase of the first pollen grain cell division. She found that pollen grains with extra chromosomes had decreased viability but that some such grains can function. Petrov (97) observed the frequency of trivalents at first meiotic metaphase in triploid *capillaris* plants and found that the longest of the 3 of the 9 chromosomes always form trivalents, the next longest 3 in about 70% of the cells, and the shortest 3 in only 20% of the cells. This corresponds with his observation that number of chiasmata formed increases proportionally to the length of the 3 chromosome types, although the regularity of this is interfered with by the tendency of chiasmata to be spaced at regular intervals. He also observed that the average number of chiasmata formed by the 2 longest homologues varies in different diploid plants of *C. capillaris;* in plants homozygous for translocations, bivalents are formed regularly; but in plants heterozygous for translocations, the chromosomes involved usually form chains. In the latter category of plants the shorter chromosomes may form no chiasmata and may be present as univalents, giving rise to daughter nuclei with different numbers of chromosomes (*cf.* 102, 103).

The first report on chiasma formation in *Crepis* was by J. Clausen (26) who found chiasmata in more than 50% of the pollen mother cells. One such cell of *C. aspera,* a 4-paired species, appeared to have 5 chiasmata, one in each of 3 pairs and 2 in the fourth. A study of the shape of the conjugants at diakinesis in *Crepis* showed that the shape of the bivalents has a certain relation to the location of chiasmata. More recently, Jenkins (55) reported that in each of 4 species of *Crepis,* all with $n = 4$, there is frequently a single non-terminal chiasma in each bivalent at early diakinesis, the minimum required to maintain pairing. At late diakinesis, there was usually 1 chiasma, sometimes there were 2, rarely 3 chiasmata. "This is rather surprising in view of the great length of the *Crepis* chromosomes. Another curious fact is that there was little terminalization until late diakinesis or early meta-

phase." The general resemblance between the chromosomes of *Crepis aurea* and *C. rubra,* two 5-paired species which are well differentiated taxonomically, was noted by Koller (56). Bivalents of the two species were more or less alike at first meiotic metaphase, a combined result of similar position of the centromere and difference in terminalization. The definite difference in initial chiasma frequency and degree of terminalization shows that, while the chromosomes of these two distantly related species are rather similar in appearance, they must have important internal differences. The significance of chromosomal changes in the evolution of *Crepis* has been discussed.

Meiosis in the pollen mother cells of the sexual, diploid forms of *Crepis acuminata* and *C. occidentalis,* both of them American species, was found to be normal by Stebbins and Jenkins (120). But in tetraploid *C. occidentalis* and in *C. intermedia* (an assemblage of allopolyploids), meiosis in the pollen mother cells is characterized by the presence of 2 to 7 multivalents and 1 to 4 univalents. In these polyploid forms, 30 to 40% of the male sporocytes show chromatin bridges and fragments at first anaphase, indicating chiasma formation in inverted segments. Aside from such irregularities, meiosis in the pollen mother cells is completed normally. But, as Stebbins (118) points out, in the facultatively apomictic groups there is no evidence of correlation between the percentage of apomictic development and amount of abnormality in the pollen mother cells. This is particularly clear in *Crepis* (120) where an attempt was made to obtain such evidence. "The meiotic abnormalities in the male and female cells must therefore be considered as separate phenomena."

Meiotic Metaphase Pairing in Hybrids

The earlier evidence on irregularities in chromosome distribution in the first meiotic division in interspecific hybrids in *Crepis* was reviewed (16) with the conclusion that the genus is a group of more or less closely related species. The major subgroups, recognized by earlier systematists, *Catonia, Eucrepis* and *Barkhausia,* are not well differentiated but overlap more or less, and numerous hybrids have been produced between species of different major subgroups. Eleven interspecific hybrids were studied (1, 16, 18, 50, 74) with reference to the amount of pairing at first metaphase.

The evidence indicates that the genic complements of the 14 species involved are all more or less homologous. This inference is consistent with the evidence on chromosome morphology in the genus (14, 17) ; on geographic distribution (9) ; and on the results from genetic investigations on small groups of closely related species (15, 55). The evidence on metaphase pairing in interspecific hybrids, therefore, supports the conception that the species of *Crepis* had a common origin and are still more or less similar in genetic constitution. Inference of antecedent structural changes in the chromosomes of species from meiotic behavior in interspecific hybrids is discussed in the next section.

Interspecific Hybridization as an Evolutionary Process

Indirect evidence. Experimental evidence on the origin of new species of *Crepis* through interspecific hybridization is largely of an indirect nature. But, when all this evidence is taken into consideration, the writer is convinced that this process is secondary in importance to gene mutation and chromosome changes in this genus. In the first place, there is some indication, based on comparative morphology and karyotypy, that certain groups and certain individual species of *Crepis* actually originated through hybridization. The 3 species with $n = 7$, have already been mentioned. Another group of species with $n = 6$, of which *C. pygmaea* is the most primitive member, was considered (14) as probably to have originated through hybridization between 5-paired ancestors. Now that 6 is recognized as a primitive number in *Crepis,* it seems more probable that the 2 or more parental species had 6 or more pairs of chromosomes. Two other closely related species which may have arisen through hybridization between primitive *Crepis* and primitive *Hieracium* species are *C. paludosa* and *C. viscidula*. The former is one of the most widespread of all the Old World species, and it is unique in having certain characteristics that are strongly *Hieracium*-like. The latter is endemic in Bulgaria and is typical of *Crepis*. From karyotype analysis, Babcock and Swezy (22) concluded that *Crepis biennis,* an octoploid with $n = 20$, originated as an amphidiploid hybrid between two species with $n = 5$ and later doubled its chromosome number. Still another Old World species, *C. syriaca,* has variable chromosome numbers, and it was suggested by Cameron (24) that most probably it originated through hy-

bridization between two different races of its close relative, *C. alpina,* followed by chromosomal alterations in the progeny. Finally, the hybrid origin of the 7 diploid American species with $n = 11$ may be said to be as definitely established as is possible without direct experimental proof.

Despite all this indirect evidence, however, it must be admitted that the number of *Crepis* species for which origin by hybridization is definitely or strongly indicated by available evidence is relatively small. Experimental evidence on the creation of potential new species by hybridization of existing species of *Crepis* is still more limited. Except for one case (*C. neglecta × C. fuliginosa*), discussed below, *Crepis artificialis* is the only experimentally produced form which could be considered a potential new species. As stated above, the original, supposedly constant form has broken down into several distinct races with different chromosome numbers, some of which may yet prove to be constant. But the origin of *C. artificialis* was possible only because one of the parents is a polyploid. Such species are very rare in *Crepis*. Hence, it is the rôle which hybridization plays, as an initial step in making *interspecific* structural changes possible, which makes it next in importance to intraspecific structural changes as an evolutionary process in *Crepis*. Considerable experimental evidence has accumulated which demonstrates the importance of this rôle of interspecific hybridization.

Navashin (*cf.* 18), in his early work (1927) on *Crepis capillaris × C. aspera,* observed structural changes in one of the *capillaris* chromosomes and inferred that this change had probably occurred at meiosis in the F_1 hybrid. Later, the same author (83) reported that chromosomal rearrangements are greatly increased in *Crepis* hybrids; and still more recently, he (87) reports observations, in progenies of species crosses, of chromosomes which have been changed morphologically and presumably also structurally.

Evidence from structural hybridity. Müntzing (74) studied meiosis in a hybrid between *C. divaricata* and *C. Dioscoridis,* both species having $n = 4$ but belonging in rather widely separated sections of *Crepis.* Besides an average of only 1.8 bivalents at first metaphase in the pollen mother cells, fragments were observed at diakinesis and metaphase, and in some first anaphase figures there were chromatin bridges and fragments, representing double attachment chro-

mosomes. Müntzing concluded that the chromosomes of the two species have homologous segments, and that a somewhat different position of these segments in the pairing chromosomes would cause the bridges and fragments through crossing over. Various alternative arrangements of the homologous segments could produce bridges and fragments, and fragments might also arise from association of non-homologous segments at pachytene. These observations demonstrate a mechanism capable of giving rise to chromosomal alterations of evolutionary value.

In this connection it is pertinent to note that Sveshnikova (121), working on *Vicia*, was able to reproduce, in a hybrid between *V. sativa* and *V. amphicarpa*, the translocation which had given rise originally to a certain small-sized chromosome in *V. sativa* and which distinguished that species from its wild relative. She concludes that such translocations may point to the presence of phylogenetically connected chromosomes in a group of species.

Both spontaneous and induced translocations were observed in the chromosomes of interspecific hybrids by Gerassimova (44). As a result, new chromosome types were formed, resembling certain chromosomes which are typical of other species. Among the F_2 and F_3 progenies of 5 different *Crepis* hybrids, chromosomal alterations were found which resulted from translocations occurring during sporogenesis. Interspecific translocations may also arise under certain conditions in somatic tissues of hybrids, and here their number is limited only by the number of ultimate chromomeres. But translocations occurring during sporogenesis would be determined by the number of conjugating chromomeres and, in hybrids forming no bivalents, may not occur at all. Hence, translocations in somatic tissues may be of the greater potential importance in plants. *Thus, interspecific hybridization combines with mutation* (structural change) *to form an important additional factor in evolution, at least in the evolution of Crepis.*

Two similar investigations on interspecific *Crepis* hybrids are now being completed in the Division of Genetics of the University of California. Miss Sherman (unpublished) has studied meiosis in F_1 hybrids between *C. Kotschyana* (= *C. Bureniana* in Babcock and Cameron, 14), with $n = 4$, and 6 other species all with $n = 5$. Like most other 4-paired species, *Kotschyana* lacks the small V-shaped "E" chromosome which is present in its 5-paired relatives.

On morphological grounds, as well as from geographic distribution, there was good reason to place *C. Kotschyana* in a section containing the six 5-paired species mentioned above. Hence this seemed to be promising material in which to obtain cytological evidence of chromosome homology and possibly to throw some light on the origin of *C. Kotschyana*. Regarding the first possibility, expectations have been abundantly fulfilled. In all hybrids, chromatin bridges and fragments have been found, similar to those reported by Müntzing in *C. divaricata* × *C. Dioscoridis*. Regarding the manner of origin of *C. Kotschyana* no conclusion has yet been reached, but the evidence for partial genetic homology between its chromosomes and those of its supposed near relatives is in itself a strong indication that the 4-paired *Kotschyana* was actually derived from some 5-paired ancestor or ancestors, presumably by a process involving reciprocal translocations.

Evidence from karyotype analysis. Our other investigation, by H. A. Tobgy (unpublished), deals with *Crepis neglecta, $n = 4$, C. fuliginosa, $n = 3$,* both F_1 and F_2 hybrids, and certain hybrid forms found in nature. A study of meiosis in F_1 hybrids revealed definite evidence of the existence of homologous segments in the chromosomes of the two species. The *A* and *D* chromosomes of *neglecta,* through unequal translocation, gave rise to the *A* and *D* chromosomes of *fuliginosa;* while the *B* and *C* of *neglecta,* through a similar interchange of segments, gave rise to the *B* chromosome of *fuliginosa.* One arm of the *neglecta C* chromosome and its centromere are absent from the *fuliginosa* complement. Hence it may be inferred that the 3-paired *fuliginosa* has been derived from *neglecta,* or from a common 4-paired but now extinct ancestor, through a process involving chromosome interchange and resulting in reduction from 4 to 3 pairs of chromosomes, as well as in marked change in karyotype. Because of the structural differences in the parental species, the F_1 hybrids were highly sterile; but with persistent effort it was possible to obtain F_2 and back-cross progenies totaling 64 plants, among which were found both parental and F_1 types, as well as numerous new forms with chromosome numbers ranging from 6 to 11. One of the F_2 segregants was similar in morphology and karyotype to a certain wild plant, grown from seeds collected by Miss S. P. Topali in northeastern Thessaly where it is known that the two species have come into contact. This particular form

is about 70% fertile. It has a *neglecta* karyotype, but one or more chromosomes carry *fuliginosa* segments which explains the presence of certain *fuliginosa* characters. The occurrence of a duplicate of this wild form among the F_2 segregants provides the best clue to the method of origin of intergrades occurring in nature. In addition to the above form, which was produced by 2 successive cross-overs in the same arm, several new forms with changed karyotypes, resulting from single crossing-over, have been obtained among the F_2 progeny. This direct evidence on the origin of a 3-paired species from a 4-paired ancestor is just as outstanding, in the opinion of the reviewer, as Miss Gerassimova's demonstration of the origin of intersterility within a species through chromosomal transformation.

These two investigations indicate the basic importance of structural changes in karyotype evolution in *Crepis* and, like those previously reported, they show the secondary importance of interspecific hybridization as a process of origin of new species through interspecific translocations leading to entirely new types of plants with new types of chromosomes.

It may be objected by some taxonomists, however, that only a very few naturally occurring interspecific hybrids have been reported in *Crepis*. Although only some half-dozen such hybrids have been named and described, yet there is a mass of experimental evidence showing that interspecific hybrids can be produced and that they often occur spontaneously. There is also the evidence, reviewed above, that hybridization occurred several times during the history of the genus. Furthermore, on account of physiological isolation in new forms produced through hybridization and consequent structural changes, the morphological intergrades which once existed as a result of hybridization would tend to disappear in course of time. Hence a relative scarcity of natural hybrids at the present time would have little bearing on what may have happened during geological epochs.

POLYPLOIDY AND APOMIXIS IN CREPIS

The Occurrence of Polyploidy in the Genus

Polyploidy of all sorts is very rare in *Crepis*. Among those which have been discovered, 4 have been so little studied that their classification as auto- or allopolyploid is purely conjectural. One

of these is *C. polytricha,* a tetraploid perennial with $x = 4$, and a close relative of *C. chrysantha* of northeastern Asia.   It differs from *chrysantha,* however, in certain morphological characters and so may be an allopolyploid.   Another, *C. incana,* is one of the few pink-flowered species and is a high montane perennial from southern Greece.   It is a tetraploid with $x = 4$ and it has no closer relatives than the next.   The third is *C. taygetica,* a suffrutescent, yellow-flowered, alpine species, endemic in the Taygetos Mts., of southern Greece.   It has $2n = ca.$ 40 and, from the limited material available and the crowded condition of the chromosomes, it was not possible to ascertain definitely whether its base number is 4 or 5 nor to work out an idiogram of the karyotype.   That *C. ciliata,* with $2n = ca.$ 40, is an octoploid, was shown by Babcock and Swezy (22) from karyotype analysis, and it is sufficiently close to *C. biennis* to be placed in the same section.   Unlike *biennis* which is of wide distribution, *ciliata* is confined to the Caucasus region. Furthermore, its karyotype does not indicate that it was of amphidiploid origin; but meiosis has not been studied in *ciliata,* and without further evidence it should not be assumed to be an autopolyploid.

In the complex, polymorphic species, *C. vesicaria,* 3 of the subspecies, *viz., typica, taraxacifolia* and *myriocephala,* are mostly diploid, but natural tetraploid forms occur which from their morphology certainly appear to be autopolyploids.   One subspecies of *C. vesicaria,* however, *viz., stellata,* appears on the same grounds to be an amphidiploid, originating from a cross between other subspecies of *vesicaria.*

Another tetraploid species from eastern Asia, *Crepis crocea* with $x = 4$ (first reported as *C. Bungei* 2174 by Hollingshead and Babcock, 53), appears to be an amphidiploid, derived from a hybrid between *C. Bungei* and *C. oreades,* both with $n = 4$.   That *C. biennis* ($x = 5$, $2n = ca.$ 40) is probably a doubled-up amphidiploid was explained above.

The American Species of Crepis

With the exception of *Crepis nana* and *C. elegans,* which have $n = 7$ chromosomes and belong in a section containing 5 Asiatic species, all of the native American *Crepis* have the base number, $x = 11$.   It was proposed by Babcock and Navashin (18) and Hollingshead and Babcock (53) that this group arose as amphi-

diploid hybrids between Asiatic or extinct American species with lower chromosome numbers, probably $n = 4$ and 7. This hypothesis has been strongly corroborated by the monographic work on the group by Babcock and Stebbins (21) and Stebbins and Babcock (119). Ten species with the base number 11 are recognized. One of these, *C. runcinata,* consists of 7 geographic segregates or subspecies, all with the same chromosome number $(2n = 22)$. It occurs widely in the western United States, the center of its distribution being the central Rocky Mountains. There is no evident connection between it and the other 9 species. The latter include 7 diploids with the somatic chromosome number $2n = 22$. These, taken by themselves, are entirely distinct from one another, but they are connected by a continuous, complex series of intergrading, polyploid forms, partly or wholly apomictic, with somatic numbers ranging from 33 to 88. The polyploids are of 2 sorts: a few are morphological autopolyploids, identical with the diploids except for their *gigas* characteristics; the great majority are allopolyploids, which combine the characteristics of 2 or more diploids.

Except for *C. runcinata,* each diploid form is confined to a single climatic province or part of one; 6 occur in northeastern California and adjacent Oregon; 2 in central Washington, 1 of these extending into southern British Columbia. The autopolyploids usually do not occur outside the province occupied by the corresponding diploid. The allopolyploids show by their distribution the combination through hybridization of the physiological characteristics that determine their distribution. The different forms have different soil preferences, so that their distribution is partly governed by the occurrence of different geological formations, as shown by intensive studies of small areas in northeastern California.

These species with $x = 11$ were probably not all derived from the same 4- $\times$ 7-paired hybrid, since each of the different diploids finds its counterpart in one or two species of eastern Asia, with $n = 4$ or $n = 7$. After the amphidiploids reached North America, 2 processes began. First, they hybridized to produce more or less sterile progeny, and at the same time they may have produced autopolyploid offspring. Second, by means of chromosome doubling in the diploid $(2n = 22)$ F_1 hybrids, or by hybridization of autopolyploids of two different species, or between the autopolyploid of one species and the diploid of another, the various intermediate allopolyploids

were produced. The subsequent evolution of the group has been determined by hybridization, polyploidy and apomixis, coupled with the selective effects of the environment.

To cover this and similar groups of interrelated species containing polyploids, the concept of the heteroploid (chiefly polyploid) complex is defined and developed. Genetically, such a complex can be distinguished as either sexual or agamic. Compared with homoploid groups, such complexes show great variability and taxonomic diversity, particularly in the regions occupied by 2 or more diploids. The application to agamic complexes of the usual criteria on which the species concept is based indicates that there are in these complexes no entities that are homologous to species, as they exist in homoploid sexual groups. Hence, a systematic treatment of this type of complex is proposed in which species and subspecies are recognized chiefly on the basis of the distinctions between the diploid sexual forms. A large number of formae apomicticae which have no taxonomic status are described to cover the individual biotypes perpetuated by apomictic reproduction.

Apomixis and Evolution

This analysis of the apomictic species of *Crepis* is an attempt to correlate the recent discoveries of cytogenetics with the patterns of variation found in nature and to estimate the significance of these patterns in evolution. Both polyploidy and apomixis have operated to cause great differentiation within this group of *Crepis* species; but these species are not all equally likely to persist. In some of them the diploid $(2n = 22)$ form is dominant and aggressive in most parts of its range; whereas in others it is restricted in distribution and these species are destined to become "senescent." The ultimate fate of an agamic complex of which the sexual ancestors have become restricted or extinct can be predicted; it will flourish as long as conditions remain favorable, but it will be unable to meet new changes in the environment and will therefore in time become more and more restricted and will eventually die out. It is clear, therefore, as Stebbins (118) concludes, that apomixis is not a major factor in evolution, however important it is in increasing the polymorphism and geographic distribution of the genera in which it is found.

Stebbins and Jenkins (120) investigated aposporic development

in the American species of *Crepis*. They found that reproduction in the polyploid forms of these species was by means of somatic apospory followed by diploid parthenogenesis. The frequent presence of chiasma formation in inverted segments in the polyploids, along with its absence in the diploids, is cytological evidence of the allopolyploid nature of the former; and this agrees with the evidence from the morphological appearance of the forms investigated. Megasporogenesis and formation of the embryo sacs in the diploid sexual forms is normal in every respect. The polyploid apomicts may be grouped into 3 classes in respect to the frequency of apospory and the time at which it begins. In the majority of these forms apospory occurs in 78 to 87% of the ovules, and usually begins while the megaspore mother cell is still in prophase. Embryo formation begins in the apomicts before the buds open, and is preceded by rapid division of the endosperm nuclei. The great hydration of the aposporic cell, which affects first the cytoplasm and then the nucleus, suggests that the mechanism of somatic apospory involves a change in water relationships at the chalazal end of the nucellus. Most of the *Crepis* apomicts are of hybrid origin (allopolyploids), but hybridization is probably an accompanying phenomenon rather than the cause of apomixis. The presence of predominantly apomictic reproduction, together with the occasional production of segregating and hybrid types by means of the sexual process, accounts very well for the pattern of variability found in these species.

SUMMARY AND CONCLUSIONS

The genus *Crepis* is an outstanding group of plants for the following reasons:

1. It is a natural group because all the evidence points to more or less genetic homology among the species and to their common origin and center of distribution.

2. The genus includes a remarkable range of morphological types, extending almost continuously from primitive to advanced. The primitive species are those possessing several or all such features as a perennial root and suffrutescent or woody caudex; large lyrate-pinnatifid or nearly entire leaves; tall robust stems or, in alpine species, scapiform stems; few large flower-heads; the involucre poorly differentiated into outer and inner series of bracts or

with very large outer bracts, and the inner bracts remaining unchanged at maturity; large florets; large, very coarsely beaked or fusiform achenes; and coarse, stiff pappus-bristles. In marked contrast, the most advanced species are small, very precocious, annuals; with small, often dissected leaves and low slender stems, bearing many small heads; the involucre with extremely few small outer bracts and the inner bracts specialized by cortical thickening; very small florets; very small achenes with a fine, delicate beak; and very fine, soft pappus-bristles. Between these two extremes there exist series of groups (sections) of species exhibiting various degrees of advancement or specialization; and within many such groups there is a similar range from more primitive to more advanced species.

3. Along with this morphological evidence for progressive evolution within the genus, an orderly progression is found in modification of chromosome number and morphology. The evolutionary series of chromosome numbers is 6, 5, 4, 3 among the diploid Old World species. The small section with $n = 7$ is not as primitive as the 6-paired species and requires a special hypothesis to explain its origin. Nevertheless, there is good evidence that the most primitive 6-paired species of *Crepis* were derived from the same ancestral stock as the primitive 8-paired species of *Lactuca, Prenanthes* and *Youngia*. The trend of modification in the *Crepis* karyotype is from nearly uniform, more or less medianly constricted chromosomes to distinctly different types of chromosomes, with few or none having median centromeres. At the same time there has been a general trend, with certain exceptions, toward a marked decrease in length of the chromosomes. In general morphologically similar species have similar karyotypes, again with certain exceptions, and this fact has been helpful in determining taxonomic classification.

4. Experiments with hybrids have revealed various degrees of genetic homology between species in the same and different sections; and this evidence has been helpful in determining degrees of relationship between species. Interspecific hybridization has also thrown much light on the nature of the genetic processes involved in the evolution of this large group of plants.

5. Four different processes of genetic change have been recognized (18, 14, 7) as playing more or less important rôles in the

evolution of *Crepis*. These processes, in the order presented in this review are: *a*) structural transformation of the chromosomes; *b*) interspecific hybridization; *c*) gene or point mutation; *d*) polyploidy and apomixis.

The question of the relative importance of the four processes, in making progressive evolution possible in this genus, involves a number of considerations. Structural chromosomal transformations produce an *initial intraspecific sterility which makes possible* the accumulation of further intersterility along with morphological and physiological divergence. Therefore structural changes in the chromosomes must be recognized as a type of genetic change of primary importance in this genus. Directly associated with the remarkable degree of karyotype modification in *Crepis* is the morphological evidence of progressive evolution from primitive to advanced types of plants. But a great deal, or possibly all, of this morphological reduction and specialization may be due to "gene or point mutations" which have accumulated before or since the initial karyotype changes took place.

Again, the fact that 5-paired species are at present more numerous than 6-paired species, and that the 4-paired species are much more numerous and generally more aggressive than the 5-paired species, indicates an important relation between reduction in chromosome number and the present distribution of the species. Furthermore, it is practically certain that reduction in chromosome number from 4 to 3 has occurred on 3 occasions, *i.e,* in 3 different 4-paired ancestors. From the great divergence among the several sections containing both 5-paired and 4-paired species, it is highly probable that reduction from 5 to 4 also occurred in several lines of descent; and the change from 6 pairs to 5 pairs probably occurred more than once. But these species in the various chromosome-number groups, as we know them today in their various niches and areas, fit into their respective environments no doubt partly, if not wholly, as a result of gene or point mutations and natural selection.

Gene or point mutations have been shown to play a definite rôle in the accumulation of intraspecific sterility such as characterizes in some degree, the incongruity between species. Since this category of genetic change is omnipresent and general in its effects on the organism, it must be recognized as of basic importance in evolution.

It is well to keep in mind, moreover, the evidence, already mentioned, indicating genotype control of chromosome length and width; also the possibility that there are "innate tendencies" in certain individual plants toward the occurrence of structural changes in the chromosomes. These phenomena may depend upon such simple genetic differences as would fall into the general category of gene or point mutations. If it can be proved that "genic" differences determine or condition susceptibility to the occurrence of gross structural changes in the chromosomes, such as large reciprocal translocations, then genovariation must be recognized as the more basic evolutionary process. This is a deserving field for future investigation. At the same time there exists the probability that gene mutations cause both the progressive chromosome shortening and the concurrent reduction in size of the plant and its parts (as well as reduction in length of the life cycle) which, in a general way characterize *Crepis* evolution.

For the present, it is sufficient to acknowledge that *both* gene or point mutations and gross structural changes in the chromosomes have been of primary significance in the evolution of *Crepis*. Interspecific hybridization has also been of importance but it is definitely secondary to the other two. Polyploidy and apomixis have played a very definite but relatively unimportant rôle in the evolution of *Crepis*.

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THE DIPLOID CELL AND THE DIPLOIDISATION
PROCESS IN PLANTS AND ANIMALS, WITH
SPECIAL REFERENCE TO THE HIGHER
FUNGI—CRITICISM AND REBUTTAL

Under date of October 6, 1941, Miss Mary Noble, referring to Dr. Buller's review which appeared in the July and August, 1941, issues of The Botanical Review, wrote as follows:—

"I wish to draw attention to certain misstatements which have been made therein concerning my work on *Typhula Trifolii*.

"On page 365 Buller states, 'In 1937, Noble, in fixed and stained preparations of *Typhula Trifolii,* made observations confirmatory of those of Lehfeldt. But neither Lehfeldt nor Noble studied living mycelia. . . .' On page 69 of my paper it is stated that 'Kniep's agar film technique, as modified by Sass (1929), was used for the study of the vegetative hyphae both in the living condition and after fixing and staining' and, again, page 93 . . . 'for critical work it is essential that the hyphae should be fixed and stained after examination in the living condition. . . .'

"Continuing the sentence first quoted from page 365 of Buller's paper the contention is made . . . 'and they do not seem to have been aware that, as Wahrlich (1893) had shown for the Higher Fungi in general including the Clavariaceae, there is an *open pore* in the centre of each septum, so that there is protoplasmic continuity from cell to cell.' On pages 76 and 77 of my paper this point is discussed at some length providing ample evidence that I, if not Lehfeldt, was quite cognisant of the literature concerning septum formation in the Higher Fungi.

"I would be obliged if a correction could be made concerning these misrepresentations."

Doctor Buller's reply to this criticism was solicited by the editor of the Review. His response was as follows:

"Miss Noble, in her paper on 'The Morphology and Cytology of *Typhula Trifolii* Rostr.', said: 'Kniep's agar film technique, as modified by Sass (1929), was used for the study of the vegetative hyphae both in the living condition and after fixing and staining' and also 'for critical work it is essential that the hyphae should be fixed and stained after examination in the living condition.' These statements were duly noted by me before writing my review and were regarded as being true for many of Miss Noble's observations but as not applicable to the question at issue, namely, is there or is there not an open pore in each septum of the mycelium of the Higher Fungi including the species of Typhula?

"I re-discovered for myself (*Researches,* Vol. V, Chap. II, 1933) the presence of an open pore in the septa of such Higher Fungi as *Fimetaria fimicola, Gelasinospora tetrasperma, Pyronema confluens, Ascophanus carneus, Ciboria* sp., and *Rhizoctonia solani (Corticium solani)* solely by observing the granular cytoplasm (and in some species vacuoles) moving through the septal pore from one living cell to another toward growing points. Miss Noble makes no mention of this method and presumably, therefore, she did not attempt to employ it.

"In the living hyphae of several Higher Fungi, *e.g., Pyronema confluens* and *Rhizoctonia solani,* I watched the development of individual septa, witnessed their ingrowth from the wall of the parent hypha (*cf.* the closing of the substage diaphragm of a microscope) and observed, by the flow of the cytoplasm from the penultimate to the ultimate hyphal cell, that the septa never became complete but, on ceasing to grow, were left each with a central open pore (1933). Miss Noble gives no indication in her paper that she attempted to repeat these observations with her Typhula.

"In living hyphae of *Pyronema confluens, Gelasinospora tetrasperma,* and other Higher Fungi, I observed that, when a hypha is elongating at its tip, labile cytoplasm passes from the penultimate cell into the ultimate cell by flow through the open pore of the septum separating the two cells (1933). Miss Noble, apparently, made no attempt to repeat these observations with *Typhula Trifolii.*

"Also, in living hyphae of *Coprinus sterquilinus* and *C. lagopus,* I observed a number of successive stages in the development of single clamp-connexions (1933). Miss Noble makes no mention of having attempted to study the living clamp-connexions of *Typhula Trifolii* in this way.

"Miss Noble did not illustrate the microscopic appearance of the living mycelia of her Typhula. Nowhere in her paper does she represent living cells with their cytoplasm and vacuoles, but all her drawings of hyphae show nuclei which, as is well known, can be seen only in preparations that have been killed and suitably stained.

"In the paragraph complained of I made no reference to Miss Noble's knowledge of the literature concerned with septal pores; and I still feel that the statement 'Neither Lehfeldt nor Noble studied living mycelia and they do not seem to have been aware that, as Wahrlich (1893) had shown for the Higher Fungi in general including the Clavariaceae, there is an *open pore* in the centre of each septum, so that there is protoplasmic continuity from cell to cell' is substantially correct. Wahrlich's important paper (worthy of translation as a classic) is not included in Miss Noble's literature list, and she cites him only at second hand from my Volume V. She does not mention that Wahrlich observed septal pores not only in the Ascomycetes, Gastromycetes, Ustilagineae, Uredineae, Tremellineae, Agaricaceae, Polyporaceae and Hydna-

ceae, but also in the *Clavariaceae*. It would indeed be remarkable if septal pores and protoplasmic continuity existed in the genus Clavaria but not in the genus Typhula.

"Notwithstanding Wahrlich's excellent illustrations of pores in the septa of the clamp-connexions of *Coprinus atramentarius* and *Merulius lacrymans* (reproduced in my Volume V), Miss Noble, arguing from work done by Strasburger in 1880 on Saprolegnia (one of the Lower Fungi), says: 'Therefore there is evidence that, since septa of a clamp connexion are laid down in young hyphae filled with protoplasm, they are formed according to Strasburger's 'simultaneous' method in which no pore occurs; and sufficient evidence has not been offered to substantiate the assertion that pores are always present in the septa of the higher fungi'. From this citation it seems clear that, whatever Miss Noble's knowledge of the literature concerned with septal pores may have been, she was far from being aware that such pores are generally present in the septa of the Higher Fungi.

"Miss Noble fixed and stained her preparations with a view to observing nuclei in the hyphae of her Typhula, but makes no mention of employing Wahrlich's technique (staining the cytoplasm intensely with iodine, swelling the septal walls with chlor-zinc iodine, *vide* my Vol. V, pp. 89–91) devised for the purpose of observing septal pores and the continuity of the cytoplasm from cell to cell. With Wahrlich's technique, I have observed the septal pores and the continuity of cytoplasm from cell to cell in *Fimetaria fimicola, Rhizoctonia solani* (*Corticium solani*) and the dermatophyte, *Microsporon filineum* (Vol. V, Figs. 54 and 55). As soon as Wahrlich's technique comes to be applied to a study of the structure of the septa of Typhula species, it seems most likely that septal pores and protoplasmic continuity will be found there.

"In conclusion, it may be remarked that in Higher Fungi having wide hyphae and granular cytoplasm, the existence of septal pores and the passage of labile cytoplasm through them can be demonstrated by means of photography. My former pupil, Dr. E. S. Keeping of the Provincial Laboratory, Edmonton, Canada, showed at the Third International Congress for Microbiology held at New York in 1939 (*Report of Proc.*, 1940, p. 541) moving pictures of growing hyphae of *Gelasinospora tetrasperma* in which cytoplasm could be seen 'streaming toward the tips of the hyphae and passing through the perforations in the transverse septa'."

CONSERVATION OF SCHOLARLY JOURNALS

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

THE DESERT VEGETATION OF NORTH AMERICA

FORREST SHREVE

Carnegie Institution of Washington

INTRODUCTION

The word "desert" is used in a geographical sense and in a biological sense. The former usage is commonly applied to extremely dry regions which are poor in plant and animal life. Students of the biological features of arid regions have been led to a somewhat broader use of the word.

It is possible to designate a series of desert regions, commencing with those that are extremely dry and barren and progressing by easy stages through those with slightly more favorable moisture conditions and more vegetation, to the deserts with at least one distinctly favorable season and with relatively well developed vegetation. Such a series of deserts presents the same unity as a series of forest types, between which there are, nevertheless, some important differences.

The physical conditions of a natural area merge gradually into those of adjacent areas, except where an abrupt topography quickens the change. Each of the vast continental deserts merges into more favorable regions which are not to be regarded as desert, although they may have features which relate them to it.

The geographical conception of desert is not devoid of consideration of the vegetation but lays stress upon a number of important physical features. These include: low and uncertain rainfall, high percentage of sunshine, low atmospheric humidity, high diurnal air temperature, great daily range of temperature, very high surface soil temperature, intermittent streams, active erosion by water and wind, high salt content of soil, and many minor features related to these. The economist's definition of desert is the one found in the dictionaries: "any region in which irrigation is essential to permanent agriculture." This is merely a simple expression of one of

the consequences of desert conditions rather than a definition of them.   Such conceptions of desert picture it as a background for life.   It is obvious, however, to the biologist that desert must be distinguished by the life which prevails there and not at all by the physical conditions which are encountered nor by the devices required for human occupation.

An attempt to distinguish and define types of vegetation involves dealing with complicated entities which differ in a variety of ways. If the effort is made to include animal as well as plant life in the analysis, the problem becomes still more complicated.   The difficulty lies mainly in the large number of criteria that may be used and the differences in the stress that may be laid on each of them. The vegetation of the world differs amazingly from place to place. In the study of it there are certain principles of general application. Also there are many cases in which sound generalizations drawn from the study of one type of vegetation are not true of another distant and highly dissimilar type.   The widely accepted view that there are certain "fundamental units" of vegetation is itself open to question.

The many-sided character of vegetation gives it no one adequate and satisfactory basis for placing all of the types in a linear or a dendritic scheme of arrangement, such as is given to the classification of species by their phylogenetic relationship.   The study of plant succession has led to much satisfaction as affording a "genetic" scheme of relationship between communities.   As a matter of fact, the successional relationships are merely sequential and in no sense genetic.   They result from secular changes of habitat conditions, and from the reaction of the vegetation upon its habitat, and are, therefore, tied up with the whole extraneous field of the physical conditions which control vegetation.   The recognition of successional stages serves merely to link together communities which have already been distinguished and defined on their intrinsic characteristics.   It is these intrinsic characters which afford the only basis for the recognition of types of vegetation.

In the study of any body of vegetation three fundamental questions arise: What kinds of plants does it contain?   How are they associated?   What is their taxonomic position?   Adequate answers to these questions will give a complete characterization of the vegetation, and will make it then possible to determine its geographical

range, its habitat location, its successional relations, and its controlling physical conditions Elementary logic demands that none of these extrinsic matters should enter into the recognition and characterization of the body of vegetation. The literature on vegetation amply shows that classification has rested primarily on the answers to the three questions above given. These define the anatomical-physiological types—or life-forms—of plants involved; the physiognomy, structure and sociological features displayed; and the floristic composition. With a triple standard it is still difficult to classify vegetation and to avoid laying unequal stress on the three sets of criteria. The task is greatly simplified, however, if attention is confined to the three sets of intrinsic features.

During the past ten years there has been little attention given to the life-forms of plants. In the study of the community and its social organization there has been a large body of good work. Floristic composition has long received adequate attention. In much ecological work it has been given, in fact, more emphasis than it merits in that connection.

DESERT VEGETATION

In terms of the three criteria that have been mentioned, desert vegetation may be distinguished by its physiognomy and life-forms, by its structure and social organization, and by its floristic content. The desert is rich in plants which are peculiar in habit and structure. The communities of the desert are low, open and either very regular or very irregular in the pattern of their organization. The flora of the desert is very distinct as to its species but strongly related to adjacent regions as to its genera.

Life-Forms of the Desert

In most of the great vegetations of the world a single life-form prevails in the uppermost layer of the dominant plants, even if it be represented by a large number of unrelated species. This life-form may be the evergreen needle-leaved tree, the deciduous tree, the sclerophyllous shrub, the grass, or any one of several other forms. Desert vegetation, on the other hand, is characterized by the presence among its dominants of a considerable number of life-forms. These plants often represent wholly distinct types of adjustment to their environment. They have originated and survived in a vege-

tation in which there is not the keen competition and resulting standardization that is found in the denser vegetations of moist or wet regions. It is true that in desert communities of simple composition the density of stand is largely determined by root competition. In mixed stands there are usually such great differences between the habit of growth, seasonal activity and root distribution of the several life-forms that their representatives are not competitors for water or light, even if they grow very close together.

The recognition of life-forms is based upon the fact that the vegetative characters of plants are largely independent of their phylogenetic relationship. The prolonged interest in life-forms is due to their importance in determining the physiognomy of the vegetation, and to their close relationship to the conditions of climate and soil.

The first scheme of life-forms was proposed by Humboldt (1806) and was an outgrowth of the observation on his wide travels that the appearance of vegetation was greatly influenced by the presence or absence of evergreen trees, deciduous trees, palms, bamboos, cacti, aloes, grasses, *etc*. More extended systems of life-forms were proposed by A. P. DeCandolle (1818), Kerner (1863), Grisebach (1872) and Hult (1881). In all of these systems, except that of DeCandolle, the sole objective was grouping the types of plants according to their rôle in determining the physiognomy of the vegetation. After Darwin the vegetative organs of plants began to be viewed from the standpoint of their "adaptive" and selective value. New systems of life-forms were then set forth by Warming (1884), Reiter (1885), Drude (1887) and others, in which the physiognomic significance was subordinated to the physiological. Drude then led a movement to disregard purely morphological features (Organizationsmerkmale) and to recognize only the adaptive features (Anpassungsmerkmale). This tendency reached its climax in the system of Raunkiär (1904 *et seq*.) which is so simple and easily employed that it has replaced all of the older schemes and has almost brought an end to further philosophical consideration of life-forms. This system has been used to determine the "biological spectra" of the floras of various regions, these being merely tabulations of the percentages of the several categories of the system which are present in the entire flora. Such tabulations have never been attempted for other systems of life-forms. There has been some well grounded criticism of Raunkiär's system because it

is based on a single set of characters—those having to do with resting over unfavorable seasons—and is, therefore, a classification of plants in their resting rather than their active phases. The modification and extension of Raunkiär's system, published by Braun-Blanquet (1932), is a very rational and useful one, although it is still open to the criticism just mentioned.

The history of the various life-form systems has been very fully related and discussed and a full bibliography given by Du Rietz (1931), who then proposes a system of his own based primarily on the size and vegetative type of plants during their active period. A more recent discussion of life-forms was published in this journal by Adamson (1939), who is impressed by the lack of agreement in viewpoint and terminology, which is such an outstanding feature of all ecological topics. Adamson concludes that the life-form systems of Drude and Du Rietz are so complicated that they are difficult of application "or, like that of Warming, have the drawback of being based upon more than one criterion" (*l.c.,* p. 549). There are indeed few cases in which a single criterion is acceptable for any biological classification, and in which ease of application may be allowed to become a guiding principle.

The study of desert vegetation is calculated to awaken interest in life-forms. The types of plants are so unlike, their rôle in the physiognomy of the vegetation is so strong, and the relation of their gross structure to the environment is often so clear, that the study of these types becomes an important phase of desert ecology.

When attention is directed to the life-forms of the North American Desert it is at once obvious that a general system, of world-wide applicability, is not required for a natural expression of the array of obvious types. Many important life-forms are absent, and many others are restricted to uncommon habitats, at the same time that several forms which are uncommon elsewhere are so diversified as to demand subdivision.

Adamson (*l.c.*) has brought together the biological spectra for several widely separated deserts, as based on Raunkiär's life-form classes. Comparison shows little agreement among the desert spectra, which means that Raunkiär's classes do not afford a natural expression of the vegetative diversity of desert plants, and that the percentages in which they are represented do not express a relation to the climatic differences of the principal desert areas. These limitations are particularly potent when the spectra are based

on the *flora* rather than the *vegetation,* and the rare plants are thus given as much weight as the abundant ones.

In the course of work on the North American Desert the writer has developed a very simple classification of the life-forms which are abundant or conspicuous in this area. The scheme is as follows and is in the form of a key indicating the distinguishing features on which the subdivisions are based.

LIFE FORMS OF THE NORTH AMERICAN DESERT

Ephemerals
 Strictly seasonal
  Winter ephemerals .... 1. *Daucus, Plantago*
  Summer ephemerals .... 2. *Pectis, Tidestromia*
 Facultative perennials .... 3. *Verbesina; Baileya*
Perennials
 Underground parts perennial
  Perennial roots .... 4. *Pentstemon, Anemone*
  Perennial bulbs .... 5. *Allium, Hesperocallis*
 Shoot base and root crown perennial .... 6. *Hilaria, Aristida*
 Shoots perennial
  Shoot reduced (a caudex)
   Caudex short, all or mainly leafy
    Leaves succulent .... 7. *Agave, Hechtia*
    Leaves non-succulent .... 8. *Nolina, Dasylirion*
   Caudex long, leafy at top
    Leaves simple, semi-succulent .... 9. *Yucca*
    Leaves branched, non-succulent ...... 10. *Inodes, Washingtonia*
  Shoot elongated
   Plant succulent (soft)
    Leafless, stem succulent
     Shoot unbranched .... 11. *Ferocactus, Thelocactus*
     Shoot branched ....
      Shoot poorly branched ....
       Plant erect and tall .... 12. *Pachycereus, Carnegiea*
       Plant erect and low or semi-
        procumbent and low .... 13. *Pedilanthus, Mammillaria*
      Shoot richly branched
       Stem segments cylindrical .... 14. *Cylindropuntia*
       Stem segments flattened ...... 15. *Platyopuntia*
    Leafy, stem not succulent .... 16. *Talinum, Sedum*
   Plant non-succulent (woody)
    Shoots without leaves, stems green .. 17. *Canotia, Holacantha*
    Shoots with leaves
     Low bushes, wood soft .... 18. *Encelia, Franseria*
     Shrubs and trees, wood hard
      Leaves perennial .... 19. *Larrea, Mortonia*
      Leaves deciduous
       Leaves drought deciduous
        Stems specialized
         Stems indurated on surface 20. *Fouquieria*
         Stems enlarged at base ... 21. *Bursera, Idria*
        Stems normal
         Stems not green .... 22. *Jatropha, Plumeria*
         Stems green .... 23. *Cercidium, Euphorbia*
       Leaves winter deciduous
        Leaves large .... 24. *Populus, Ipomoea*
        Leaves small or compound .. 25. *Olneya, Prosopis*

This classification is merely an adaptation of some of the older ones and is based on the same kinds of criteria that have been used by Warming, Drude, Du Rietz and others.   The most important of these are: duration of life, height, character of stem, amount and manner of branching, the presence or absence of leaves, the insertion, size and seasonal duration of leaves, presence or absence of succulence in stem or leaf, *etc.*   No attempt has been made, or can be successfully made, to distinguish Organizationsmerkmale and Anpassungsmerkmale, since they are equally important in reference to physiognomy, and the former are often of such a character that they have been useful in enabling their possessors to take a place in desert vegetation.   Inherited morphological limitations have done everything to give *Yucca* and *Agave,* for example, their characteristic forms, but at the same time these plants have both gross and minute structural features which make them very successful desert plants.

The series of life-forms here outlined is not of general applicability, neither is it complete for the flora of the North American Desert.   The omissions include submerged and emergent aquatics, parasites, saprophytes, epiphytes and vines.   All of these types are represented but are not important constituents of the vegetation. Vines are locally abundant in the southern part of the desert but their root and leaf characters are more important than the scandent habit of their stems.   The great diversity of form that is shown by the succulents and their importance in the vegetation require their subdivision.

The life-forms which are most abundant and of most general occurrence throughout the North American Desert are: nos. 1, 3, 4, 6, 7, 8, 9, 11, 13, 14, 18, 19, 20 and 25.   The ones which outnumber all others are: nos. 14, 18, 19 and 25.   Forms definitely restricted to the warmer parts of the desert are: nos. 10, 21 and 22.   The poorest representation is found in the northernmost part of the desert and the richest in the low elevations of the southernmost part.   In any one region the greatest uniformity of vegetation is found on the broad plains, on which the soil has uniformity of surface, depth, texture and moisture content.   In such situations the number of associated life-forms may be reduced to three or four. In contrast, the greatest variety is found on coarse outwash slopes and the lower slopes of hills and mountains, where the conditions

of soil, soil moisture and atmospheric features are complex. The number of life-forms to be found closely associated under such conditions ranges from 15 to 21 of the total series of 25.

Structure of Desert Vegetation

The structure and social features of vegetation are more simple in the desert than in moist regions. The plant communities of the North American Desert are far from being uniform in their structure, organization and social characteristics. These features are strongly influenced by climate and by soil and surface conditions, which are everywhere closely related to the topography. Also the structure is greatly affected by the life-forms which are present in a given region, particularly the small desert trees, large columnar cacti and leaf succulents. It seems very probable that historical factors thus affect the structure of the vegetation, just as they do the physiognomy and the floristic composition. Strong contrasts in structure are found between the northern and elevated parts of the desert and the southern parts at low altitude, and also may be found in different topographic sites in the same region. The life histories of the large perennials and of the ephemerals and herbaceous perennials are so different that they require separate consideration in reference to their social behavior.

It will be convenient to treat separately the pure or nearly pure stands, better designated as "simple stands," in which two or three species are dominant, the mixed stands, in which dominance is shared by 4 to 12 species, and the rich stands, in which the highest development of desert vegetation is found.

The simple stands of the broad plains display the greatest uniformity of structure as well as the greatest simplicity of composition. It may be estimated that about 12% of the North American Desert is occupied by extensive monotonous communities of this character, in which two or three species form 90 to 95% of the population and a single species determines the physiognomy of the community. The coverage is from 8 to 15%, with the open spaces broader than the spread of the plants. The simplicity of composition results in a uniformity of height such as is not found elsewhere in the desert. This is very noticeable in the extensive areas of *Larrea divaricata, Artemisia tridentata* and *Coleogyne ramosissima.* The height of the simple stands varies with the soil moisture supply.

Height and density are closely correlated. It may be noted in many localities, however, that the tallest stands of *Larrea* and *Artemisia* are as pure as the low ones.

In the region dominated by *Artemisia tridentata* the commonest subdominants are species of *Chrysothamnus* and *Atriplex,* in the widely separated parts of the desert dominated by *Larrea divaricata* the subdominants are *Franseria dumosa, Encelia farinosa* or *Flourensia cernua.* In each of these cases there are differences between the dominants and subdominants in root distribution or leaf behavior which greatly reduce competition for water between them. Their dissimilar requirements are further exemplified by the frequency with which the subdominants occur in habitats in which the dominants are absent.

The uniformity of height and density in the simple stands tends to obscure the dissimilarity in age of the individual plants. This is manifested in the size of the basal crown from which the branches rise, or the absence of such a crown in young plants, by the size and number of branches, by the number of depressed dead branches, and by the smooth level soil surface under younger shrubs as compared with the hummock from which the older plants very frequently rise. Reproduction is very poor in undisturbed stands. In *Larrea* it is not possible, on the level plains, to find more than one seedling from two to five years old for every 400 mature plants. If the old plants are cut off or removed, or if the surface is broken or overturned, seedlings will appear in large numbers and the survivals will re-establish a stand, at first denser than the original one but finally identical with it.

Destruction of the original vegetation results in the appearance of seedlings, which not only constitute the first stage of succession but include in their number the individuals which will ultimately restore directly the original vegetation. It may be said that successions do not take place in desert vegetation and they cannot, therefore, be "telescoped." It is at least certain that the succession concept would never have been developed in a study of the vegetation of arid regions. One of the principal reasons for the abeyance of successional phenomena is the almost total lack of reaction by the plant on its habitat. The existence of a plant in a given spot for many years does nothing to make that spot a better habitat for some other plant or some other species. Fallen leaves and twigs

are blown away, or small accumulations of organic matter are washed away, to be carried ultimately to the nearest flood plain or playa. Also little happens to change the character of the soil or its water-holding capacity. Only in the rich stands of desert vegetation is there a local amelioration of conditions due to the presence of large and long established perennials.

Mixed stands of desert vegetation are defined as those in which the dominance is held by 4 to 12 large perennials. This slight enrichment of the composition involves plants which differ so much in life-form, height and spacing that the mixed stands form a sharp contrast to the simple ones. Approximately 60% of the North American Desert consists of mixed stands. They occupy the nearly level plains in which there is a coarse or stony soil, the long gently sloping outwash plains, or bajadas, and the hills or mountain slopes in which the conditions for retention of moisture are not the best. There are usually broad transition belts between the simple and mixed stands.

In all but the northernmost parts of the desert mixed stands include groups of species which all, or nearly all, belong to different life-forms. The "canopy" of the community is, therefore, broken into several levels and the individuals of the same height are so widely spaced that the profile is very uneven. Microphyllous evergreen or deciduous shrubs form three or four levels, scattered trees rise to two or three times the height of the largest shrubs, while large succulents or semi-succulents rise above the level of the trees. The tallest plants assume an importance in the physiognomy of the vegetation out of all proportion to their numbers.

The even spacing of the simple stands is here replaced by a marked inequality. This is quite haphazard, resulting in groups of varying density and composition. Plants differing greatly in height are often closely associated, while all those of the same height, considered by themselves, are nearly as widely spaced as in the simple stands. The open ground in the mixed stands is correspondingly irregular in pattern. The coverage ranges from 15 to 30%.

Mixed stands occupy situations with slightly better moisture conditions than the simple ones. The growth of individual plants and the changes in the population are, nevertheless, very slow (see Shreve, 1917, and Shreve and Hinckley, 1937).

In mixed stands the occurrence of large well established perennials, such as *Prosopis, Cercidium* or *Olneya,* provides small areas in their shade in which the conditions are slightly more favorable than in the open (Shreve, 1931). In addition to light shade and the longer retention of soil moisture, the surface of the soil under trees is covered with litter from the tree and the dead herbs of previous years. Although the duration of the litter is too brief to increase materially the humus content of the surface soil, it serves to check the movement of wind blown seeds, to conceal seeds from birds, and to give protection to seedlings during their early critical stages of existence. The area under a tree, is, therefore, commonly occupied by many more seedlings and young plants of large and small perennials than are found on an equal area in the open. The stands of ephemerals are also denser and taller in these locations. Areas immediately surrounding the trunks of large succulents and semi-succulents, such as *Pachycereus, Lemaireocereus, Yucca* and *Dasylirion,* casting little shade and dropping no litter, are as bare of young plants as the open areas. The localization of favorable conditions for seedlings results in development of the clumps of perennials which are frequent in the mixed stands and reach their maximum development in the rich stands of the southern lowland desert.

In the nearly pure stands the minimal area—the smallest in which all species of the association occur—is determined by the spacing. In mixed stands the minimal area is often not more than 100 sq. m. In several adjacent areas of this size the lists of species present are identical over areas many square miles in extent, at the same time that the relative abundance of the species may or may not vary greatly. Long distances may be traversed in the simple or mixed stands without discovering additions to the species represented on the initial 100 sq. m. area. The species which are limited to flood plains, to the banks of streamways, to exceptionally shallow soil, to highly alkaline areas, or to other special habitats, likewise recur with constancy and great uniformity.

The ephemerals and herbaceous perennials, active only in the favorable seasons, are controlled by physical conditions and social relations very unlike those governing the perennials. The coming and going of the stands of ephemerals, their allocation, density, height and composition are determined by the varying chances of

rainy seasons, surface soil conditions and size and dissemination of seed crop. The sociological features of the ephemerals, which are seasonally and locally such a conspicuous element of desert vegetation, are little different from those of other regions in which conditions for them are more favorable or of longer duration.

If a given region is observed from year to year it will be noted that the amount and time-distribution of the seasonal rains determines the ephemerals which appear in greatest abundance. In southern Arizona copious and frequent winter rains result in great abundance of *Lupinus, Streptanthus, Eschscholtzia* and *Thelypodium,* while late and scanty rains lead to great abundance of *Plantago, Chaenactis, Festuca* and the introduced *Erodium.* Throughout the desert the ephemerals of both winter and summer occur in scattered colonies of great density, at the same time that they are infrequent or absent elsewhere, a circumstance which is probably due to the manner of dissemination of the last seed crop and the weather conditions during and immediately following its dissemination.

Desert ephemerals are characterized by speed of germination, rapid growth under favorable conditions, and early maturity. A period of five to six weeks suffices for the life cycle of many species, but will not suffice for the species just mentioned as confined to the wettest seasons.

The size of the plant at the appearance of the first flowers may vary within wide limits. A seedling which is overtaken by a waning moisture supply will produce a single flower at a height of 1 to 2 in., while an individual of the same species with a good and sustained supply of moisture will branch richly, reach a height of 15 or 20 in. and produce hundreds of flowers. These extremes of development may sometimes be found within a few feet of each other.

The rich stands of desert vegetation are so designated only in contrast with the simple and mixed stands. The characteristics of the rich stands are: greater density, a large number of life-forms, more diversity in height, more layering, and the occurrence of a larger number of species. The coverage varies from 40 to 70%. In some regions the coverage is very irregular on account of the grouping of the plants, areas with nearly complete coverage alternating with areas in which it is as low as it is in the simple stands.

The number of dominant and sub-dominant perennials varies from 15 to 25, and the number of infrequent species is greater than in the mixed stands.   The minimum area must here be enlarged to a 500 meter square.

Rich stands are found in the southern part of the desert below an elevation of 800 m. in Baja California and Sonora.   With less pronounced characteristics they occur on the plateau of Mexico at elevations of 5000 to 6000 ft.   In northern Sonora and southern Arizona they are confined to habitats which are favored by water supply, soil, or slope exposure.   In the northern arm of the North American Desert they do not occur, although stands of simple composition may reach equal height and density.

The rich stands at low elevations include nearly all of the lifeforms that have been listed above.   The most abundant are small drought-deciduous trees, evergreen and drought-deciduous shrubs, large and small succulents, semi-succulents, evergreen semi-shrubs and leaf succulents.

The shade of old trees here affords favorable conditions for germination and early growth in a more marked degree than in the mixed stands.   This subarboreal layer includes young plants of large perennials as well as small perennials and vines.   Many species may be found which do not occur in the open.   So dense does the layer become that it begins to serve in itself as a favorable screen for the establishment of new plants.   The subarboreal layer then begins to extend in every direction beyond the shade of the tree.   It may coalesce with other similar mottes of vegetation or its edge may remain sharply defined by the very open matrix of shrubbery in which it has developed.

It is obvious that in these dense stands there is a degree of competition which does not exist elsewhere in the desert.   The seedlings of large nonsucculent perennials have an advantage in competition with herbaceous plants and small perennials in their very early development of a deep root system.   Noteworthy in this respect are *Prosopis, Jatropha, Olneya, Erythrina, Ceiba* and *Simmondsia*.   Succulent and semi-succulent plants (cacti, agaves, yuccas) are also exempt from competition for water, since they renew their internal supply through superficial roots at times when the surface moisture supply is abundant.

The rich stands which are found at higher elevations and in the

middle latitudes of the North American Desert are poorer in life-forms than those just described. Evergreen and deciduous shrubs, semi-shrubs, arborescent cacti and large semi-succulents are the dominant plants. The coverage varies from 30 to 60%. The canopy is relatively uniform except for the tall yuccas and large arborescent cacti. The number of common perennial species may be as great as 25 or 30, but is often less. The absence of relatively large trees does much to simplify the vegetation and to make it more uniform. The rich stands of the highlands have much in common with the mixed stands, but far exceed them in density and in the number of component species.

Floristic Composition of Desert Vegetation

In describing the structure of the vegetation much has been said on previous pages to indicate the simplicity of composition which is typical among the perennials of all but the southern margin of the North American Desert. Dominance is held throughout this vast area by a relatively small number of species which are confined to the desert or extend only short distances beyond it in the most arid habitats of adjacent regions. Several of the important dominants have large areas of distribution and are accompanied by different groups of associates in the widely separated parts of their ranges. Prominent among these are *Larrea divaricata, Artemisia tridentata, Fouquieria splendens, Prosopis chilensis* and *Acacia Greggii*. A number of genera are chiefly confined to the desert and are represented by two or more species, either respectively characteristic of different sections of the desert or else overlapping in area. The most ubiquitous of these genera are *Ephedra, Opuntia, Yucca, Acacia, Cercidium, Jatropha* and *Franseria*.

When the floristic composition changes from one locality to another the physiognomy and structure of the vegetation usually change also. The rôle of a single prominent species in giving character to the vegetation may be very strong, even if it is not one of the most abundant species. This is particularly true of plants well above the normal height of their associates, for example, *Pachycereus Pringlei, Carnegiea gigantea, Idria columnaris, Yucca valida, Ipomoea arborescens* and *Yucca australis*. The existence of varied life-forms is everywhere responsible for the profound change in the structure of communities that is often due to very slight changes in floristic composition.

Cases in which abundance is shared by two or more species of the same life-form are uncommon over most of the desert. They occur in the north, where low bushes are associated which have foliage not definitely deciduous but subject to deterioration during the winter. These are exemplified by species of *Atriplex* and *Franseria* and by *Grayia spinosa*. Also in the south there are a number of microphyllous winter-deciduous shrubs which form the matrix of the vegetation in certain localities, particularly limestone hills. The commonest are *Caesalpinia gracilis, Acacia vernicosa, Coursetia glandulosa, Mimosa laxiflora, Rhus microphylla, Cassia Wislizeni* and *Phaulothamnus spinescens*.

Members of the same genus which have the same life-form may have overlapping ranges but are rarely occupants of the same habitats. This is illustrated by *Cercidium microphyllum* and *C. floridum, Jatropha spathulata* and *J. cordata, Acacia paucispina* and *A. Greggii, Franseria deltoidea* and *F. dumosa*. Among the cacti there are many exceptions to this generalization, in platyopuntias and in the large and small globose forms.

Of particular interest are the cases in which the physiognomy and structure of the vegetation of two localities are closely similar at the same time that the dominant and many of the subdominant species are different. These cases of communities which are alike in their life-forms and their structure but different in floristic composition may be widely separated or may be contiguous in places where there is an abrupt change of altitude. Just above the local upper limit of extensive areas of *Larrea* in southern Arizona may be found similar nearly pure and evenly spaced stands of *Mortonia scabrella,* which is similar to *Larrea* in height, general habit and perennation of leaves. In localities in northern Sonora the hills and coarse outwash slopes just above the altitudinal limit of *Larrea* are dominated by nearly pure stands of *Dodonaea viscosa,* which also forms communities with close resemblance to those dominated by *Larrea*.

There are many cases in which the same ecological niche is filled in widely separated areas by members of the same life-form. These are commonly different species of the same genus, but their specific characters play little or no part in their rôle in the vegetation. Prominent examples are the tall arborescent yuccas of which *Y. brevifolia* occurs in the Mojave Desert, *Y. valida* in central Baja

California and *Y. australis* in Coahuila and San Luis Potosi. Other examples are *Prosopis chilensis* in Texas, Chihuahua and Coahuila and *P. velutina* in Arizona and northern Sonora; *Carnegiea gigantea* in Arizona and Sonora and *Pachycereus Pringlei* in Baja California; *Franseria deltoidea* in Arizona and *F. chenopodiifolia* in Baja California; *Agave cerulata* in Baja California and *A. falcata* in Coahuila. There are areas in Sonora dominated by *Larrea, Carnegiea gigantea, Cercidium microphyllum, Opuntia fulgida* and *Jatropha cordata* which bear a closely similarity in physiognomy and structure to areas in Baja California dominated by *Larrea, Pachycereus Pringlei, Cercidium peninsulare, Opuntia cholla* and *Jatropha cinerea.*

GEOGRAPHICAL AREA OF THE NORTH AMERICAN DESERT

Some aspects of the desert region are best understood in connection with its geographical extent and in the comparison of its various parts.

Although there has been some elasticity in the use of the word "desert," it is of interest to note that there is a fair agreement as to its area and boundaries among the plant geographers by whom it has been mapped. The early map of North America by Drude (1887) is floristic rather than vegetational. Schimper (1903) published the first map of the vegetation of the world from the modern ecological standpoint. His map of North America is small and very general. His indication of the north and south extent of desert in the United States, Baja California and Sonora is fairly good. He indicates two isolated areas in the Rio Grande valley and in Texas, which are extended too far north. Most of the desert on the plateau of Mexico is designated "xerophilous woodland." Harshberger (1911) was the first to present a vegetation map of North America, far more satisfactory in scale and details than the map of Schimper. His demarcation of the desert was well done and his subdivision of it into "Great Basin Region including the Oregon, Nevada and Mohave deserts," "Sonoran Desert Region" and "Chihuahuan Desert Region" has been substantiated by later work. Maps of larger scale and greater detail were published by the present author (1917) and by Shantz and Zon (1923). These maps are in good agreement except that Shantz and Zon rely on the use of "indicator plants" for their areas, and

therefore extend the "sagebrush desert" of the Great Basin north into Washington and east into Wyoming almost to the limit of the range of this plant, irrespective of its associates near its margin. They also extend the "creosote bush desert" of Texas down the Rio Grande valley to the Gulf of Mexico, although the valley below Laredo is neither desert nor a part of the range of creosote bush.

The vegetation of Mexico was mapped by Sanders (1921) who recognised "desert, including alkaline wastes" and "scrub, chiefly mesquite, yucca, agave, cactus." The area of these two categories covers all of the Mexican desert but also includes part of the thorn forest of the west coast, most of the arid bushland of northeastern Mexico, and most of the grassland of the plateau.

A very small map of the biotic areas of northern North America prepared by Shelford, Jones and Dice (1926) distinguishes "extreme desert," "desert" and "semi-desert grassland." The nature of the map scarcely permits careful reading of boundaries but indicates the occurrence of desert from eastern Oregon to San Luis Potosi.

On a very small scale, as part of a map of the world, Rübel (1930) shows the area of "siccideserta," extending from Oregon to Sinaloa and southern Tamaulipas, with five small isolated areas in the United States. This area agrees with that shown on previous maps, except in the unwarranted addition of the five isolated areas.

Some of the agreement between successive maps of the vegetation of North America is possibly due to partial dependence by each cartographer upon the work of his predecessors. The agreement must be taken at its face value, however, and indicates that the North American Desert is an area with generally recognised boundaries, and that there is, therefore, a strong unanimity of opinion among plant geographers as to the character of the vegetation designated as desert.

In an attempt to map the desert in detail it is found to be like any other great natural region in being without sharp boundaries. The severe conditions of its interior gradually ameliorate away from its center, and continue to ameliorate far beyond its margin. Placing its boundary is endeavoring merely to connect the localities which exhibit the same stages of transition from arid to semiarid vegetation. Not only is the transition usually very gradual

but it is often regressive. After leaving the characteristic land-
scapes and vegetation of the desert they may reappear, as a result
of local rainfall conditions, slope exposure or character of soil.

THE
NORTH AMERICAN
DESERT
AND ITS
SUBDIVISIONS.

GREAT BASIN DESERT

MOJAVE DESERT

SONORAN DESERT

CHIHUAHUAN DESERT
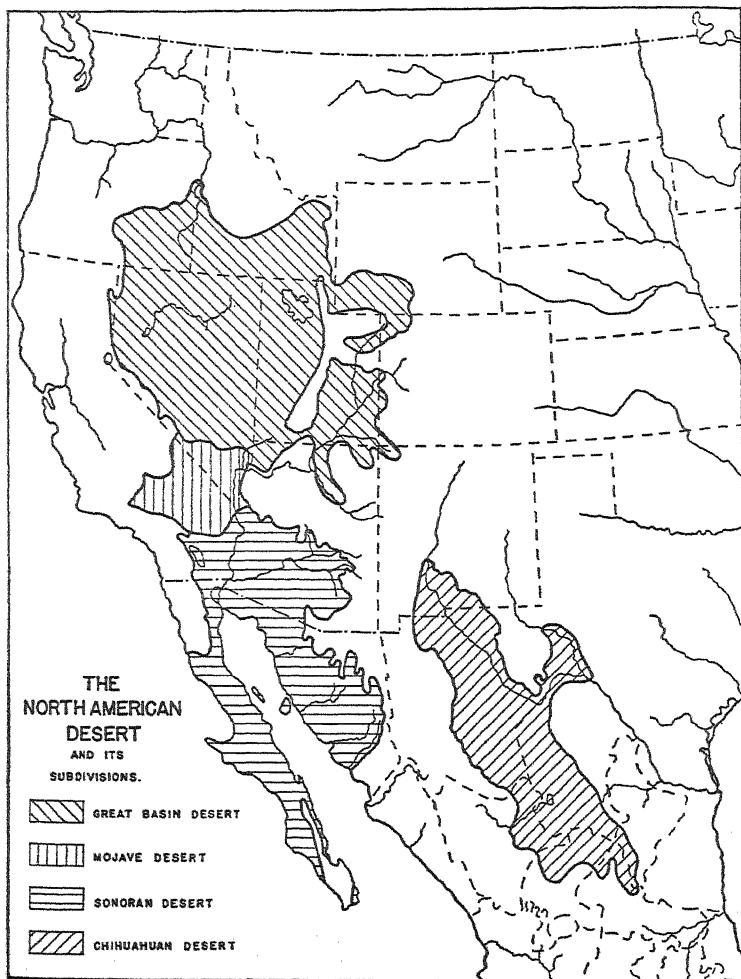
The area occupied by the North American Desert, as under-
stood by the writer, is indicated on the accompanying map. Be-
ginning at the north it will be seen that desert extends southward
from central and eastern Oregon, embracing nearly all of Nevada

and Utah except the higher mountains, into southwestern Wyoming and western Colorado, reaching westward in southern California to the eastern base of the Sierra Nevada, San Bernardino and Cuyamaca mountains. From southern Utah the desert extends into northeastern Arizona at the same time that it occupies the western and southwestern parts of that State. Desert extends south along the eastern coast of northern Baja California, in the lee of the Sierra Juarez and Sierra San Pedro Mártir. South of these ranges it extends across the peninsula as far south as the northern end of Sierra Giganta. Southward from that place it is limited to the Pacific coast, a very narrow strip along the Gulf coast and parts of the lower elevations in the Cape Region. On the mainland it occupies the lowlands of Sonora as far south as the delta of the Yaqui River.

On the highlands of southeastern Arizona and southern New Mexico the continuity of the desert is broken by Desert-Grassland Transition. At a slightly lower elevation it reappears in the valleys of the Rio Grande and Pecos rivers, extending as far east in Texas as the lower course of Devil's River. South of the International Boundary desert extends continuously through eastern Chihuahua and nearly all of Coahuila, being broken only by a few higher mountains and elevated areas of grassland. Further south desert is confined to eastern Durango, northern Zacatecas, the western margin of Nuevo Leon, and the northern part of San Luis Potosi.

In the north an isolated area of desert occupies part of the Columbia River basin in eastern Washington, while in the south several detached areas occur in Hidalgo and Puebla, notably the valleys of Ixmiquilpan, Actópan, Mesquital and Tehuacan.

Around the periphery it merges into other types of vegetation which vary greatly in their own character and in their ecological and floristic relationships to the desert. These have been briefly discussed elsewhere (Shreve, 1941). Each of the neighboring plant formations exhibits some features of similarity to the nearest parts of the desert, thereby increasing the difficulty of placing a sharp boundary to the desert region. The chaparral of California and Baja California differs from the nearest desert in its greater density of stand, in the predominantly evergreen sclerophyllous foliage, and in its strong uniformity of life-forms. The xeric wood-

land of juniper and pinyon, which borders much of the northern desert, is dominated by these trees at the same time that it has much of the openness of the desert and some of its diversity of life-forms. The grassland which approaches the desert in New Mexico, Texas and Chihuahua has a much heavier carpet of grasses than is found in any other but exceptional habitats of the desert, and is very poor in shrubs, succulents and small trees. The arid bushland which lies along the eastern edge of the desert in Texas, Coahuila and Nuevo Leon is far richer in shrubs and small trees than the adjacent desert, so that its height and density greatly exceed those of the desert. The thorn forest which lies immediately south of the desert in Baja California and Sonora is a closed or nearly closed stand of drought-deciduous and winter-deciduous trees, with some evergreen trees, large succulents and many shrubs. It has little in common with the desert except its variety of life-forms and the presence of large and small stem-succulents.

When the North American Desert is considered as a whole the predominant feature of its vegetation is shrubbery. All of the other types of plants which do so much to give character and variety to the vegetation are found, almost invariably, in a matrix of shrubs. The features which distinguish the widely separated parts of the desert are mainly due to the rôle played by small trees, the many types of stem succulents, leaf succulents, semi-succulents, sarcophytes- or fat-stemmed trees—grasses and ephemerals.

Desert shrubs fall into two rather well defined groups. Members of one have woody stems, are moderately branched and undergo a definite seasonal termination of growth, being in many respects much like the shrubs of the eastern states. Examples are *Larrea, Acacia, Caesalpinia, Cassia, Eysenhardtia, Coursetia* and *Karwinskya.* The members of the other group have soft brittle stems, are very richly branched and have indeterminate growth without formation of resting buds. Examples of these are *Artemisia, Chrysothamnus, Atriplex, Franseria, Encelia, Salazaria, Hyptis* and *Aplopappus.* The first group may be designated "shrubs" and the second "semi-shrubs." The semi-shrubs are not confined to the desert but are a very common element in its groundwork of shrubbery, decreasing in dominance from the north and the highlands toward the south and the lowlands.

The impression of monotony which is given by the vegetation

of large sections of the desert is due to the matrix of shrubbery and the fact that either one shrub is strongly dominant or that the dominance is shared by several shrubs which are similar in life-form, height and color of foliage.   The rôle in the physiognomy of the vegetation which is played by the groundwork of shubbery rests largely on the differences between shrubs and semi-shrubs. In the northern and the more elevated parts of the desert, semi-shrubs are dominant, while in the southern parts and those at lower elevations the shrubs are dominant.

Subdivisions of the North American Desert

The following pages will be devoted to a brief description of the features which differentiate the several parts of the North American Desert.   The subdivisions are based on physiognomy, community structure and floristic composition.   Underlying these ecological features are substantial differences in latitude, altitude and nearness to the sea, all influencing the climate, while there are, likewise, differences in orography, topography and mineralogy which sometimes affect large areas.   The biological features which distinguish the major subdivisions of the desert are closely correlated with differences in the existing physical conditions.   It is not yet possible to state, however, to what extent the differences in flora and floristic composition have been influenced by historical factors.   Which is merely to say that the balance between plants and environment which exists to-day may be in part a mere survival from some of the successive stages of the similar balance that has existed throughout the history of land plants.

The subdivisions which are recognised are: Great Basin Desert, Mojave Desert, Sonoran Desert, and Chihuahuan Desert.   The location and boundaries of these areas are shown on the accompanying map.   Geographical rather than vegetational designations are used for the subdivisions on account of their being simpler and more generally understood and because all of them have long been in use among plant and animal geographers.   California botanists frequently allude to the part of the Sonoran Desert which lies in their State as the "Colorado Desert."   This is a rather ambiguous name and overlooks the fact that the area in question has no features which distinguish it from adjacent parts of Arizona, Baja California and Sonora.

There have been no full and comprehensive treatments of the ecological features of any of the subdivisions of the North American Desert. The report on the Death Valley Expedition by Coville (1893) and the observations on distribution of common perennials made on the same expedition by Merriam (1893) are full of valuable data on the Mojave Desert. There are many pages relating to the United States parts of the North American Desert in general papers by Clements (1916, 1920). Local studies have been made by Hanson (1924) in northern Arizona, Graham (1937) in the Uintah Basin, Utah, and by Bray (1906) in western Texas. A number of papers have resulted from study of the slender grazing resources of the desert or of the indicator value of its natural vegetation for agricultural possibilities. Some of these give valuable information on regions which are otherwise very poorly known. Noteworthy among them are Griffiths' (1902) observations in northern Nevada and southeastern Oregon, Nelson's (1898) description of the Red Desert of Wyoming, Aldous and Shantz's (1924) treatment of the economic significance of the types of vegetation in the semi-arid portion of the United States, and a paper on the influence of grazing on plant succession in Utah by Cottam and Stewart (1940).

For the Sonoran and Chihuahuan Deserts there have been published a number of brief or local papers, including titles by Spalding (1909), MacDougal and collaborators (1914), Shantz and Piemeisel (1924), Nichol (1937) and Shreve (1934a, 1936b, 1939).

The Great Basin Desert. The Great Basin Desert is nearly coextensive with the floor of the Great Basin, and extends into adjacent parts of the Columbia Plateau and Colorado Plateau. It includes the Harney Basin in Oregon and the Snake River Plains in Idaho, extending south to southern Nevada and Utah, and reaching eastward into the Red Desert of southwestern Wyoming, the western border of Colorado and the northeastern corner of Arizona. The area lies largely above 4000 ft. in elevation and is subject to frequent periods of freezing temperatures of a week in duration. On approximately seven eighths of the Great Basin Desert the rainfall is between 4 and 8 inches, increasing to 11 and 13 inches at higher elevations and in the northern extension. The low precipitation is, however, more uniformly distributed through the year than in the other desert areas. At most localities the rainfall is

heaviest in the spring months, with June the dryest month.   The rains of midsummer are lighter, in most localities, than those of the autumn and early winter.   The absence of a protracted dry season, in conjunction with the moderate temperatures that result from higher latitude and altitude, gives the Great Basin Desert more favorable moisture conditions than the low precipitation would indicate.

As a result of the well known structure and topography of the Great Basin the floor of the desert consists of a very large number of undrained basins separated by fault-block ranges or by great accumulations of outwash material.   At the center of each basin is an alkaline flat or dry lake.   Around the flat are concentric belts in which the soil conditions gradually change on going toward the edge of the basin.   The principal changes are toward a coarser texture and a lower content of salts.   With variations in size of basin and central flat the same pattern is repeated almost throughout Nevada and Utah.

In the Great Basin Desert the salient features of the vegetation are its simplicity of composition and its fidelity of distribution with relation to the belts of soil conditions.   Over the valleys and outwash slopes are spread pure, or nearly pure, stands of semi-shrubs which often extend for 20 to 60 miles with little change.   Moving into one of the other soil belts brings a rapid transition to a different but equally monotonous community of similar extent.   Only where there is rock in place, much sand, or a broken and irregular surface is there a more varied assemblage of plants.   The two communities which are most widespread in the Great Basin Desert are those dominated by *Artemisia tridentata* and by *Atriplex confertifolia*.   The two are often associated but the latter appears chiefly in a lower belt than *Artemisia* and in more alkaline soil.

Artemisia tridentata occurs discontinuously throughout the Great Basin Desert and ranges beyond its borders in every direction, into the xeric woodland and pine forest as well as into the Mojave Desert.   *Artemisia* is often the dominant plant in localities so unlike that they might be expected to have different vegetation.   The differences in the conditions it encounters are expressed by wide variation in density, height, rate of growth and size of individual. The finest stands are heavy but rarely compact; the height is commonly 2 to 4 ft. and seldom exceeds 6 to 7 ft.   The bulk of a tall

individual is determined by the density of the vegetation in which it has matured.   The roots often penetrate to a depth of 10 ft.

The largest lake bed in the Great Basin is that of the ancient Lake Bonneville, immediately west of Great Salt Lake.   Here, as in all of the lake beds, plants are few or absent.   Kearney and collaborators (1914) made a study of the belts of vegetation in relation to the salt content of the soil near the shore of Great Salt Lake. The lake bed and margins in which the surface soil has 2.5% or more of soluble salts are occupied by low and extremely open stands of *Salicornia rubra, S. utahensis* and *Allenrolfea occidentalis.* Where the salt content is between 0.5% and 0.9% there are colonies of *Distichlis spicata* or *Sporobolus airoides*.   With a content of 0.8% the surface is occupied by a heavier stand of the low semishrubs, *Sarcobatus vermiculatus* and *Atriplex confertifolia*.   At 20 to 300 ft. above the present lake level, and in soil with 0.5% of salts, *Atriplex confertifolia* and *Kochia vestita* are the occupants, the latter being restricted to soil of heavy texture.   Beyond this belt the coarser texture of the soil and a very slight decrease in the salt content result in a pure stand of *Artemisia*.

The two plants which share the dominance of Great Basin vegetation, *Atriplex confertifolia* and *Artemisia tridentata,* are thus seen to differ in the ability of the former to succeed on more compact alkaline soils, while the latter flourishes on the lighter and less alkaline soils.   Nearly all of the land that has been brought under cultivation by irrigation or dry farming in Nevada and Utah was originally occupied by *Artemisia*.   With each of these plants are to be found other species of the same genera, differing to a greater or less extent in their life requirements, sometimes growing as subdominants, sometimes in mixed stands just outside the areas of dominance of the common species, or in other cases confined to the xeric woodland or to higher elevations.   In *Artemisia* there are 19 species in addition to *A. tridentata* which are found in more or less close association with it in the Great Basin Desert.   *Atriplex confertifolia* is accompanied in the area by 14 other native perennial species of the genus.   The two dominant species of the area have appeared in genera which comprise a considerable number of abundant and successful desert plants.

In addition to the two leading plants there are a number of semishrubs which are very important in the vegetation of the Great

Basin Desert, either in pure stands over large areas or as the dominants in association with *Artemisia* or *Atriplex*. The most important members of this group are the following:

Artemisia nova is similar to *A. tridentata* but more open in habit and not so tall. It is found on shallow rocky soil with poor water supply, and the areas which it dominates lie above those with *A. tridentata* and in the northern part of the area.

Chrysothamnus puberulus is a compact hemispheric semi-shrub rarely more than 1 foot high and usually found in widely spaced stands. It is found only on light or sandy soils with low salt content. It occurs in the central and northern part of the Great Basin. Several taller species and their varieties are common in association with other semi-shrubs throughout the area.

Grayia spinosa is a dioecius semi-shrub from 2 to 3 ft. high, with slightly succulent leaves. The ends of its branches become spiny through the arrest of growth by drought. *Grayia* is often found growing with *Artemisia tridentata, Atriplex confertifolia* and, especially in the southern Great Basin, with *Coleogyne ramosissima*.

Eurotia lanata is a semi-shrub from 2 to 3 ft. high with narrow hoary leaves. It forms extensive pure stands in many localities in which the soil is slightly alkaline, is a frequent component of the areas of *Atriplex confertifolia,* and also grows with *Sarcobatus vermiculatus*.

Atriplex Nuttallii is a semi-shrub 1 to 2 ft. high with thick firm leaves. It grows in soil with a salt content as high as 3%, and is characteristic of extensive alkaline plains throughout the eastern half of the Great Basin Desert. It is often associated with *A. corrugata* which is even more alkali-resistant.

Coleogyne ramosissima is a small-leaved evergreen shrub from 1 to 2 ft. high which is found only on rather coarse soils very low in salt content. It is absent from the northern Great Basin but forms extensive pure stands in the south, as well as mixed stands with *Grayia spinosa*.

Artemisia spinescens is a rounded semi-shrub 6 to 18 in. high which occurs alone or with other plants in moderately alkaline plains. It comes in leaf early and is almost wholly summer-deciduous. It is particularly abundant on the plains and high mesas of northern Nevada.

Several semi-shrubs are either dominant in habitats of restricted

area or occur infrequently in pure stands and more often in association with some of the species just enumerated. *Sarcobatus vermiculatus* is the most abundant of these, forming thick stands on the flood plains of smaller streamways and occurring sporadically in all situations with a somewhat alkaline soil and a deep moisture supply. *Kochia vestita* frequently forms open pure stands on highly alkaline flats. *Tetradymia spinosa* is infrequent in pure stands but common as an associate of *Artemisia tridentata* and other upland plants. *Purshia tridentata* is abundant on both sandy and rocky soils in the northern Great Basin and Columbia Plateau, and in some places forms nearly pure stands. *Ephedra nevadensis* is sporadically abundant on deep coarse soils at higher elevations and *E. Torreyana* at lower elevations.

The monotony of Great Basin vegetation is due primarily to the pure or simple stands of plants and secondarily to the fact that nearly all of its abundant plants are of the same life-form. Their only conspicuous difference is in height, form of crown and color of foliage. The color is gray or gray-green in all of the common semi-shrubs except *Sarcobatus vermiculatus* which is a deep leaf-green. Leafless green-stemmed plants are represented only by *Ephedra;* succulents by small forms of *Opuntia* and *Echinocereus;* semi-succulents by the infrequent occurrence of *Yucca.* Root perennials, ephemerals and grasses are abundant, but the only other life-forms are represented by plants of local or infrequent occurrence.

At the close of the spring rainy period herbaceous ephemerals are abundant and conspicuous. They include a large number of species chiefly in the genera *Eriogonum, Abronia, Lepidium, Lupinus, Astragalus, Euphorbia, Oenothera, Gilia, Phacelia, Cryptantha* and *Chaenactis.* At lower elevations the floor of the desert is relatively poor in herbaceous perennials but they increase in numbers and in species as the upper edge of the desert is approached. They are often a conspicuous feature of the vegetation on coarse soils and mountain slopes or on sand, in both spring and summer.

Below elevations of 5000 to 5500 ft. the slopes of hills and mountains in the Great Basin Desert are thinly covered with vegetation. At higher elevations some of the semi-shrubs of the desert floor may be found on moderate slopes in heavy stands. This is particularly true of *Artemisia tridentata.* Between 6000 and 7000 ft. hills and other situations with rock in place near the surface are char-

acterized by open stands of *Juniperus utahensis*.   The line be-
tween the shrubby desert and the arborescent xeric woodland is
often very sharp.   At the lowest elevations for *Juniperus* it occurs
as if superposed on the desert vegetation, while at higher levels,
particularly in the north, it is accompanied by shrubs and herba-
ceous perennials which are rare in the desert.

At the southern edge of the Great Basin grasses play an unim-
portant rôle in the vegetation except for the sod of *Distichlis
spicata* and the clumps of *Sporobolus airoides* in highly saline or
alkaline flats.   On the floor of the desert at lower elevations grasses
are rarely seen, perhaps the most common one being *Hilaria
Jamesii*.   In Nevada north of the Humboldt River grasses begin
to play a more important rôle.   Hills and slopes, as described by
Griffiths (1902), are clad with grasses rather than shrubs.   The
characteristic species are *Poa Buckleyana, P. Wheeleri, Agropyron
spicatum* and *Festuca ovina*.   The slopes which are thus covered
mark the limit of the desert much more definitely than those bear-
ing open stands of *Juniperus*.

Mojave Desert.   This is the smallest subdivision of the North
American Desert and lies almost wholly in California.   The name
is often used in a restricted sense as applying only to the south-
western corner of the area which is here described.   The explora-
tions carried out by the Death Valley Expedition and reported upon
by Coville (1893) were almost entirely within the Mojave Desert
in the sense of this paper, or in its mountain ranges, but Coville
continually alludes to the Mojave Desert as a distinct part of the
area which he explored.   The Mojave Desert resembles the Great
Basin Desert in its poor display of life-forms, in the simple com-
position of most of its communities, and in the strong control of
the distribution of its vegetation by the texture and salt content of
the soil.   On the other hand, it has a few conspicuous life-forms
which are not in the Great Basin, and is dominated by two plants
which are absent from the Great Basin, at the same time that all
but two of the dominants of the latter area are here reduced to a
secondary rôle or are absent.   The floras of the two regions have a
very high percentage of their genera in common, but outside the
adjacent margins there is a far lower percentage of species in
common.

The Mojave Desert lies in southeastern California, east of the

southern end of the Sierra Nevada and north and east of the San Bernardino Mountains, extending east to the Colorado River and north approximately to the 4500 foot contour line in California and Nevada. The northern boundary is very sinuous, with frequent reversions, and may be taken as corresponding with the northern limit of *Larrea*. The distribution of this shrub has been described in detail for this region by Merriam (1893). In the northern part of the Mojave Desert is the famous Death Valley, which is 135 miles long and at its lowest spot is 480 ft. below sea level. There is, however, only a small part of the Mojave Desert below 1000 ft., and about three fourths of its area lies between 2000 and 4000 ft.

The face of the Mojave Desert is covered by numerous mountains, varying in position, height and mineralogical composition. The percentage of the total area occupied by mountains is greater than in the Great Basin Desert. The presence of the great mountain ranges on the west and south is responsible for the existence of desert conditions, at the same time that the storms which break over their summits give the elevated inner margin of the desert a greater rainfall than it receives elsewhere. Only a small fraction of the Mojave Desert drains to the sea, through minor tributaries of the Colorado River. The remainder of the surface has potential drainage into large lake beds or into the innumerable small dry lakes. The Mojave River is the only important one rising in the adjacent mountains and ending in the desert in an extensive alkaline flat. The largest of the drainageways originating in the desert is Amargosa Creek, which terminates in Death Valley. As in the Great Basin there are, therefore, many bare lake beds surrounded by zones of vegetation of decreasing ability to withstand highly charged soil. The lake beds are perpetual sources of dune-building material, which accumulates near the edge of the lake or is often blown far across the outwash slopes and deposited on distant hills.

The Mojave Desert is more arid than the Great Basin Desert. The few available rainfall records indicate that only a marginal fringe receives an annual total of more than 5 in. The irregularity of precipitation which marks all deserts is also pronounced here. During a period of 12 years (1909–1921) the railway station of Bagdad suffered three rainless periods, respectively, 24, 25 and 32 months long. The aridity is accentuated by the seasonal distribu-

tion of the rain, which falls in the winter and early spring and is almost wholly lacking in the summer months. The eastern edge of the Mojave Desert is sometimes visited by the fringe of summer storms, which are increasingly frequent eastward from the Colorado River.

The southwestern edge of the Mojave Desert, which lies nearest to the centers of population of southern California, is the best known part of the area, at the same time that it is richest in its flora and the most diversified in its vegetation. Here are registered the combined effects of an elevation between 3000 and 4000 ft., a rainfall of nearly or just above 5 in., and areas of granitic loam soil of low salt content. Along the upper edge of the desert the landscape is dominated by *Yucca brevifolia,* which is stout of trunk, freely branched and from 20 to 30 ft. in height. With the *Yucca* is a heavy but discontinuous stand of shrubbery about 4 ft. high, in which are associated a larger number of species than are found in any other part of the Mojave Desert. The commonest of these are *Artemisia tridentata, Larrea divaricata, Tetradymia spinosa, Aplopappus linearifolius* var. *interior* and *Acamptopappus sphaerocephalus.*

With increasing distance from the base of the mountains the spacing of the plants becomes wider, *Yucca brevifolia* rapidly becomes smaller and less frequent, some of the semi-shrubs mentioned above disappear, and *Larrea* begins to dominate the vegetation. The largest area of *Yucca* and heavy shrubbery lies between Victorville on the east and Palmdale, Lancaster and Mojave on the west. In this area and the *Larrea* region adjacent to it the spring rains bring forth the herbaceous ephemerals and perennials which form such a high percentage of the flora of the region and give a few weeks of greenness and color to the desert.

Elsewhere in the Mojave Desert the upper bajadas which descend from the larger mountains bear an impoverished similarity to the marginal belt but this is not true of the hills and smaller mountains. Approximately 70% of the Mojave Desert is covered with an open or very open stand of *Larrea divaricata* and *Franseria dumosa,* the remainder being occupied by dry lakes and their marginal zones or by mountains large enough to bear a more diversified vegetation.

Between the marginal and central parts of the Mojave Desert may be observed one of the characteristic distinctions between the

less extreme deserts and the very arid ones. In the former it will be commonly noted that there is greater local differentiation of the vegetation than in the latter. The margins of streamways have denser stands of shrubbery than the general surface, usually including species confined to the streamways. Hills and slopes with rock in place have heavier stands than the bajadas and plains, invariably including many species which are absent from level deep soil. Slope exposure has some influence on the composition of the vegetation and a greater influence on the relative abundance of its components. Differences in soil texture are accompanied by differences in the composition and density of the vegetation, even in cases in which the amount of soluble salts is not involved. Over most of the Mojave Desert, as in other very dry deserts, these features of differentiation are absent or very weakly expressed. Throughout the central and eastern part of the area the tilted plains and rolling surface are covered with a stand of *Larrea* and *Franseria* which blankets the desert without reference to drainage, slope or soil. The only break in the monotony of the plant covering is the slight difference in stature and density that may be observed and the irregularity of spacing that commonly exists on areas of shifting sand. In many large areas *Larrea* is reduced to a height of 15 to 18 in. and the vegetation covers only 3% of the surface.

On the floor of the Mojave and Sonoran Deserts in regions where the annual precipitation is less than 6 in. the runoff is so small and its detrital load so heavy that there is no development of normal dendritic drainage. Storm water flows down the slopes in small shallow anastomosing rills, of which 20 to 30 cross a given contour level in each mile. This condition eliminates the streamway as a habitat presenting more favorable moisture conditions and does much to bring about the uniformity of spacing that may be observed from any elevated location in the desert.

Among the perennials which form minor constituents of the prevailing vegetation are *Hilaria rigida,* confined to sandy soil, *Atriplex canescens,* occurring only in somewhat alkaline situations, *Opuntia echinocarpa,* very infrequent even on stony soils, *Hymenoclea salsola,* on sand or deep loam, *Atriplex hymenelytra,* in stony soil, *Krameria pervifolia,* on coarse soil, and *Ephedra nevadensis,* on deep loam soils. On rocky slopes with thin soil the principal infrequent shrubs are *Atriplex hymenelytra, Lycium Cooperi,*

Peucephyllum Schottii, Acamptopappus sphaerocephalus var. *hirtellus* and *Dalea Fremontii* var. *Johnsonii.*

The dry lakes usually have a surface which is smooth, hard and devoid of vegetation. In many cases the prevalent *Larrea* and *Franseria* extend to the edge of the bare lake bed. In other cases there are belts of *Dondia, Allenrolfea* or *Atriplex,* or else of *Distichlis spicata* or *Sporobolus airoides.* The accumulation of sand which is commonly found on the lee side of a dry lake is partially stabilized by an irregular stand of *Aplopappus linearifolius* var. *interior, Franseria dumosa, Eurotia lanata, Hilaria rigida, Tetradymia spinosa* and, at lower elevations, half buried trees of *Prosopis chilensis.*

Areas in the center of the Mojave Desert which lie between 3700 ft. and 4000 ft. still have the aspect of extreme aridity but differ in important respects from the lower levels. *Larrea* wanes and *Coleogyne* appears, which gives the vegetation an average height of about 2 ft., the associated species become more numerous and diversified, and the density becomes greater, sometimes resulting in a coverage of 15%. In rare cases *Yucca brevifolia* appears, not always occupying all of the ground that would seem to be favorable for it. Another plant which is uncommon but conspicuous is *Yucca mohavensis,* with a simple stem of 4 to 7 ft. *Opuntia echinocarpa* and *O. chlorotica* occur with a "density" of about one plant per acre. *Franseria dumosa* persists above the vertical range of *Larrea.* Other plants which appear in the Coleogyne association are *Acacia Greggii, Dalea spinosa, Atriplex confertifolia, Salazaria mexicana, Tetradymia spinosa, Isomeris arborea, Encelia frutescens, Brickellia oblongifolia* var. *linifolia* and *Cassia armata.*

In the extreme southern tip of Nevada, southeast of the Charleston Mountains, in the low elevations of California near Needles, and in parts of Arizona west and northwest of Kingman, the vegetation is very similar to that of the Mojave Desert further west but has some characteristics of the Great Basin as well as a small representation of the plants of the Sonoran Desert. Nearly all of the larger plants characteristic of the latter area find their northern limit a short distance south of Needles, but a few of them as well as many of the herbaceous species range north on the low ground near the Colorado River nearly to Boulder Dam. Areas at 3000 ft. elevation between Chloride, Arizona, and the Colorado River are

on the tension line between *Larrea* and *Coleogyne,* with both of which *Yucca mohavensis, Acacia Greggii, Opuntia ramosissima, Eriogonum Wrightii* and *Salvia carnosa* are frequent. Along the edge of the desert north of Chloride is an extensive belt of *Yucca brevifolia* which is one of the largest and finest stands of this plant.

The typical topography, landscapes and vegetation of the Mojave Desert extend south approximately to the northern boundary of Riverside County, California, and east to the Colorado River. The transition to the Sonoran Desert, which is here regarded as comprising the Colorado Desert, takes place within a remarkably narrow belt. The change is from a treeless to an arboreal desert, from one in which succulents are rare to one in which they are abundant, from one in which *Yucca* is widespread to one in which it is of restricted occurrence, from a region with a monotonous and uniform plant cover to one with a diversified vegetation.

Munz (1935) states that about 45% of the entire desert flora of Southern California is common to the Mojave and Colorado Deserts, and that almost one fourth of the species of the Mojave Desert are confined to its borders or extend only into immediately adjacent areas. He further states that perhaps one eighth of the species of the Colorado Desert are endemic to that area. Parish (1930) stated that "of the 545 species of typical xerophilous phanerogams inhabiting the Mohave, 282 are found also in the Colorado Desert and 263 do not extend into that region." These statements agree well enough to indicate the wide floristic gap which accompanies the vegetational differences between these subdivisions of the North American Desert.

The Sonoran Desert. The Sonoran Desert occupies the lowlands surrounding the upper part of the Gulf of California, which comprise the Colorado Desert of California, the southwestern quarter of Arizona, the lowlands of Baja California and the western half of Sonora. This is a region of low relief or of plains dotted with hills and mountains. It lies chiefly below 2000 ft. and entirely below 3000 ft. The principal rivers rise beyond the desert and their waters either traverse it or add to the underflow of its flood plains. Their presence has aided in the nearly complete development of normal dendritic drainage. Excepting the Salton Sink there are very few undrained basins. Dunes and stabilized sandy plains occupy large areas near the head of the Gulf of California and smaller ones on the Pacific coast.

At low elevations the rainfall is light and uncertain. In central Baja California there are areas which have gone for four years without significant precipitation. Within 50 mi. of the shores of the Gulf of California north of Guaymas and Mulege the meager evidence indicates that the average annual rainfall is from 2 to 4 in. At increasing distances from the Gulf and at somewhat higher elevations there is a gradual increase to 12 or 14 in. Throughout the inner half of the continental part of the Sonoran Desert the precipitation is bi-seasonal, falling in late winter and in midsummer. This results in rainless periods which are much shorter than in the Great Basin and Mojave Deserts, although more exacting on account of the higher temperatures of the Sonoran Desert.

At the very low elevations approximately 75% of the surface of the Sonoran Desert is occupied by nearly level plains or very gently tilted bajadas, while at the higher elevations such surfaces form less than 20% of the area. Where the intermont plains are both extensive and very arid the vegetation bears some resemblance to that of the Mojave Desert in its monotony and simplicity of composition.

Toward the areas of higher elevation, greater rainfall and more rugged relief, the vegetation rapidly changes in character, both on the peninsula of Baja California and on the mainland, commonly resulting in the most diversified and spectacular communities of plants to be found in the North American Desert.

On the low plains which border the Colorado and Gila Rivers *Larrea divaricata* and *Franseria dumosa* are dominant, as in the lower reaches of the Mojave Desert, but are accompanied by a number of sub-dominants, including *Hilaria rigida, Opuntia echinocarpa, Acacia paucispina, Opuntia Wrightii, Atriplex canescens, Fouquieria splendens, Opuntia ramossima* and small individuals of *Prosopis chilensis.* In areas of coarse soil *Franseria deltoidea* occurs to the exclusion of *F. dumosa.* Along all of the streamways which are more than 4 or 5 ft. in width there is a heavier stand of the common shrubs and semi-shrubs as well as the small trees *Prosopis chilensis, Cercidium floridum, Olneya tesota* and *Dalea spinosa.* Changes in the character of the soil are accompanied by new plants or new composition. All of the above features serve to distinguish the most arid parts of the Sonoran Desert from the lowest parts of the Mojave Desert in spite of the identity of their dominants.

The low mountain ranges of the dryest parts of the Sonoran Desert are so bare of vegetation that they have the color of the weathered rock, which can be roughly identified at a distance of several miles. The rock crevices and canyon walls of these arid ranges support a few xeric perennials, including *Heteropogon contortus, Bebbia juncea, Trixis californica, Hyptis Emoryi, Ferocactus acanthodes, Dyssodia porophylloides, Encelia farinosa* and *Opuntia acanthocarpa.*

Between elevations of 1000 and 2000 ft. in southern Arizona and northern Sonora the communities dominated by *Larrea* and *Franseria* have been joined by numerous other associates but are confined to very level ground with great uniformity of surface and soil. At this elevation the hills and lower mountain slopes are no longer bare and the coarse soil of the bajadas which lie just below them and above the Larrea plains bear a relatively heavy cover of the type of vegetation which is distinctive of the Sonoran Desert. The salient feature of the upper bajadas and lower mountain slopes is the number of life-forms that mingle in such abundance as to indicate their equal suitability for survival under the existing conditions. The average height of the plants is greater than in any of the situations previously mentioned, and definite layering first appears. The dominance is held by *Cercidium microphyllum* and a group of columnar and arborescent cacti, including *Carnegiea gigantea, Lemaireocereus Schottii, Opuntia versicolor, O. arbuscula, O. acanthicarpa, O. spinosior* and *O. echinocarpa.* These plants do not, however, form more than 50 to 60% of the population, as the following are also very abundant:

Olneya tesota	Frost-deciduous tree
Cercidium floridum	Drought-deciduous green-stemmed tree
Prosopis velutina	Winter-deciduous tree
Larrea divaricata	Evergreen microphyllous shrub
Acacia paucispina	Winter-deciduous shrub
Opuntia Engelmannii	Flat-jointed opuntia
Franseria deltoidea	Facultative evergreen semi-shrub
Fouquieria splendens	Drought-deciduous mesic-leaved shrub
Celtis pallida	Facultative evergreen hard-leaved shrub
Opuntia fulgida	Cylindrical-jointed opuntia
Encelia frutescens	Drought-deciduous semi-shrub
Acacia Greggii	Winter-deciduous shrub
Ferocactus Wislizeni	Large barrel cactus
Simmondsia chinensis	Evergreen thick-leaved shrub
Echinocereus Fendleri	Low caespitose cylindrical cactus

In the plains of central Sonora *Larrea* becomes greatly localized and *Franseria dumosa* finds its southern limit. The vegetation is

very open but its dominants are much larger plants than in the north, the principal ones being *Olneya tesota, Cercidium microphyllum* and *Prosopis velutina.* Other small trees which are abundant in this region are *Cercidium sonorae, Fouquieria MacDougalli, Acacia occidentalis* and *Guaiacum Coulteri.* Large cacti are frequent but scarcely as abundant as in southern Arizona, including *Lemaireocereus Thurberi, Lophocereus Schottii, Rathbunia almosensis, Opuntia Thurberi* and infrequent examples of most of the species mentioned as abundant in Arizona. The ground among the small trees may be nearly bare or occupied by heavy stands of *Franseria deltoidea* or of *Encelia farinosa.* On coarse soils and low hills there is commonly an open growth of shrubs from 4 to 5 ft. in height, chiefly *Caesalpinia gracilis, Coursetia glandulosa, Jatropha cordata, Lycium Richii, Eysenhardtia polystachya, Acacia constricta* and *Paullina sonorensis.* Annual grasses are abundant after the summer rainy season but perennial species are very local. *Agave* and other leaf succulents are very rare. *Yucca* and other semi-succulents are uncommon. Both the ephemeral and perennial herbaceous plants are less abundant in numbers and fewer in species than in the Gila drainage.

The hills and upper bajadas of central Sonora are somewhat less striking in the physiognomy of their vegetation than similar situations 200 miles further north, largely because of the close stands of leguminous shrubs which are rather uniform in height and in the character of their foliage. *Carnegiea* is uncommon in the interior and *Lemaireocereus* rarely equals it in density and height. More striking and abundant than any of the cacti is the white-barked *Acacia Willardiana,* the only member of the genus in America which bears cladophylls. In Australia the majority of acacias bear these simple leaves of petiolar origin. Another noteworthy tree of central Sonora is *Ipomoea arborescens,* a winter-deciduous tree 20 to 30 ft. in height which blooms during its leafless period.

The sandy plains of extreme northwestern Sonora have a very thin plant cover and large areas support only two or three species of perennials. The winter herbaceous ephemerals are, however, very numerous in both species and individuals in this area. There is a gradual transition in the vegetation south from the sandy plains toward Guaymas or east from any spot on this coast toward the outlying mountains and foothills of the Sierra Madre Occidental.

On going inland from Tiburon Island through Hermosillo and Ures the transition is analogous to the one observed in going south from lat. 31° N. to lat. 27° N. The change is from a very open desert of shrubs, with few trees and many conspicuous large cacti to an arboreal desert with many small trees, much shrubbery, fewer cacti and a heavier growth of herbaceous perennials and ephemerals in the summer. In the 300 miles which separate the valley of the Gila and that of the Sonora River there is a very substantial change in the flora. It is doubtful that the two areas have more than one third of their species in common. This fraction, however, includes a few of the plants of ecological importance, as *Prosopis velutina, Cercidium microphyllum, Celtis pallida, Opuntia fulgida, Acacia constricta, Baccharis viminea, Krameria parvifolia* and *Hymenoclea monogyra.*

Along the inner edge of the Sonoran Desert from Arizpe and Moctezuma south to Quiriego, about 100 miles inland from the Gulf of California, the most arid habitats are strongly desert in character and the ones that are most favorable with respect to moisture conditions and protection from frost approach the density, height and composition of the drier parts of the thorn-forest of northern Sinaloa. The dryest habitats are nearly level plains and lower bajadas with soil of coarse texture, and south slopes of moderate gradient. The most favorable habitats are flood plains, canyons and small valleys which have an accumulated water supply and are at the same time protected from temperature inversions on winter nights.

The intermediate habitats of the inner desert belt of Sonora occupy a variety of situations in the rugged hills and rolling plains, where soil texture and moisture conditions are far from uniform. On low hills and slopes with coarse soil there is an open stand of *Bursera odorata, B. tecomaca* and *Jatropha cordata,* in which these trees are from 15 to 20 ft. high and separated by distances of 20 to 60 ft. They are in leaf only during the summer rainy season, and in mid September the waning foliage gives bright red and yellow hues to the landscape. Their infrequent associates include trees common to the more arid plains to the west as well as several species characteristic of the thorn forest. Irregularly placed patches of shrubbery and cacti include a large number of forms, some of which are more abundant here than elsewhere, while a few of them extend south in arid situations as far as Oaxaca. Among these are

Croton sonorae, Haematoxylon brasiletto, Acacia vernicosa, Randia Thurberi, Karwinskia Humboldtiana, Opuntia fulgida, Acacia pennatula, Encelia farinosa, Mimosa laxiflora, Condalia spathulata and *Gauzuma ulmifolia.* On the plains between the Rio Sonora and the Rio Yaqui there are several localities in which the palm *Erythea Roezlii* occurs in widely scattered stands in very open vegetation, accompanied by *Nolina matapensis* which has a trunk 6 to 10 ft. high.

On more level ground with finer soil there is a sharp contrast between the very open vegetation of the general surface and the dense thickets which border the streamways. Also, away from the streamways there are frequent mottes of denser vegetation which owe their origin to the shade of large well established trees. Where several trees are near each other the mottes have joined and spread beyond the shade. These belts and spots of denser vegetation gradually coalesce toward the south and merge into the uniform density of the thorn-forest on the coastal plain between the Rio Yaqui and the Rio Mayo. Many of the larger plants characteristic of the open desert plains range through this transition and far into the thorn-forest, as *Cercidium sonorae, Olneya tesota, Fouquieria MacDougalii, Cercidium floridum, Celtis pallida, Jatropha cordata* and *Encelia farinosa.* Throughout the southern end of the desert are found trees and shrubs which increase in frequency in the transition region and are abundant in the thorn-forest. The chief one of these is *Acacia cymbispina* which is infrequent in the most favorable habitats in the latitude of Hermosillo and is the dominant tree of the coastal plain of northern Sinaloa. Others are *Piscidia mollis, Ceiba acutifolia, Cordia sonorae, Pithecolobium sonorae, Lysiloma microphylla* and *Pachycereus pecten-aboriginum.*

Published illustrations of the vegetation of Baja California have given a distorted impression of this important section of the Sonoran Desert. There are massive plants of *Pachycereus,* heavy stands of the unique *Idria,* grotesquely misshapen trees of *Pachycormus* and several native palms. It is only in exceptional localities that any two or three of these grow together, but these spots are an irrestible invitation to the botanical photographer. At the same time there are thousands of square miles in which none of these outstanding plants occurs and in which the aspect of the vegetation is like that of Sonora or southern Arizona.

At both its northern and southern ends the peninsula of Baja California has central mountain ranges rising high enough to support mesic forest. In the north the Sierra Jaurez and the Sierra San Pedro Mártir form a nearly continuous ridge over 6000 ft. in elevation, extending from the International Boundary to Lat. 30° 30′ N. The western slopes of these mountains and the coast opposite them receive only winter rainfall, and the vegetation is a chaparral similar to that of southern California. On their east is an extremely arid coastal belt, the San Felipe Desert. The Sierra Laguna and Sierra Victoria, in the extreme south, consist of seven acute peaks and their connecting narrow ridges. From them the level-topped Sierra Giganta extends northward along the Gulf Coast to Lat. 26° 30′ N. West of the southern mountains the rainfall is very light in summer and winter. East of them it is confined to summer storms, usually light but sometimes heavy. The eastern slopes of Sierra Laguna and Sierra Victoria and their bajadas leading down to the Gulf are covered with thorn forest. West of these mountains is low, dense, arid shrubbery. There are several isolated peaks over 5000 ft. in the central part of the peninsula but only one of them, Sierra Zaragoza, has any extent of mesic vegetation.

The most arid part of Baja California is the narrow belt which extends along the Gulf coast from the delta of the Colorado River to Los Angeles Bay, in Lat. 29° N. Here there are sandy plains and dunes, stretches of poorly covered gravel, broad bands of unsorted pebbles which are subject to overflow by mountain torrents at rare intervals, and rocky slopes with little vegetation. There is little change in the physiognomy and local distribution of the plant communities from the region southwest of the Salton Sea to Guardian Angel Island. The flora also undergoes little change in these 300 miles, being very limited as to perennials and fairly rich as to winter ephemerals.

South of Rosario, in Lat. 30° N., Baja California presents a rugged topography of low elevation. The only level areas are the great point which protrudes into the Pacific toward Cedros Island, and the Magdalena Plain. The principal differentiation of the vegetation is along lines transverse to the axis of the peninsula. On almost any of these lines it is possible to distinguish, from west to east, a coastal belt subject to high winds and much fog, hills and bajadas visited infrequently by winter rains, the central axis of

hills and plateaus, and the inner slopes and bajadas with an extremely low rainfall. The vegetation changes little on going north or south in one of these belts. The flora changes constantly throughout the length of the peninsula, many of the plants by which it is augmented on going south are important factors in altering the aspect of the vegetation.

The desert faces the Pacific Ocean for about 525 miles, from just north of Rosario to a short distance south of Todos Santos. The central part of this coast is somewhat more arid than the northern and southern parts. The irregular occurrence of hills rising abruptly from the beach, narrow coastal plains, lagoons and sand dunes does much to determine the character of the vegetation. On the most exposed parts of the coast the vegetation is very low and open, on account of the almost incessant wind. Stunted shrubs as well as rocks and pebbles are covered with lichens, which give a yellow hue to some of the coastal slopes. Along and near the coast leaf succulents are very abundant, chiefly *Agave Goldmaniana* and several species of *Dudleya*. In no other part of the North American Desert is this life form so richly represented. Several species of cylindropuntia and the ubiquitous *Machaerocereus* are frequent in the shrubbery of the extreme coast. The most abundant of the shrubs are *Euphorbia misera, Lycium californicum, Franseria chenopodiifolia, Viguiera laciniata, Harfordia macroptera* and *Aplopappus venetus* var. *furfuraceus*.

In the Viscaino Desert, which occupies the great point in the center of the peninsula, much of the surface is level, hard and almost devoid of plants. Along its inner edge, however, is a heavy stand of vegetation dominated by *Yucca valida,* and undoubtedly forming the finest display of any member of this genus in the North American Desert. The Magdalena Plain receives far more than half of the drainage of the Sierra Giganta but provides few outlets to tide water. The plain is covered with thousands of small shallow depressions which are lakes for a few weeks in exceptionally rainy seasons, or in other cases are meadows thickly covered with herbaceous plants. The network of large perennials which surrounds the depressions is very irregular in its density and height. Its characteristic plants are: *Prosopis chilensis, Pachycereus Pringlei, Lycium brevipes, Opuntia cholla, Machaerocereus gummosus, Prosopis Palmeri, Fouquieria peninsularis, Euphorbia californica,*

Lemaireocereus Thurberi and *Acanthambrosia Bryanti*.   Certain small areas reach the maximum density for desert, but alternate closely with open areas, and in both of them the familiar desert types are strongly dominant.

In the rugged interior of Baja California there are few localities of the kind that favor broad stands of vegetation of uniform physiognomy and composition.   Distance from the sea is a factor of importance but its effects may be outweighed by the steepness or gentleness of the gradient presented to the ocean winds.   Elevation is of little importance except in its relation to precipitation, and this relation appears to lack uniformity.   Recent volcanic eruptives predominate over other types of surface and the soil is either a thin clay, a clay loam filled and covered with angular fragments, or scattered pockets of clay in the fissures of very young basaltic outflows. Several widely separated granitic areas furnish soil which is very different in texture, depth and water-holding capacity.   In general, but not invariably, the volcanic surfaces support a very open vegetation which is relatively poor in its plant cover, while the granitic soils have a heavier and more diversified plant covering.   The only simple communities of wide extent in the central part of the peninsula are found on a few plateaus above 2000 ft.

It would be possible to draw up a list of thirty or more large perennials which are widely represented in the vegetation of central Baja California and are locally abundant.   However, nearly all of them are limited in distribution to a certain part of the area or else confined to definite habitats.   On some of the small granitic plains almost all of these perennials are represented.   On gravelly clay bajadas *Idria* and *Agave Goldmanii* are often dominant.   In other localities there are open forests of erect and shapely trees of *Pachycormus,* or heavy stands of *Lemaireocereus* and *Pachycormus.* Some of the very young lava flows are nearly barren while others are covered with a surprisingly heavy growth of shrubs and small trees.   The relatively high interior plateaus are covered by a very uniform mixture of *Atriplex polycarpa* and *Lycium californicum.*

The drainage divide of the peninsula is much nearer the Gulf than the Pacific.   A narrow belt of very arid country extends south from the San Felipe Desert nearly to San Jose del Cabo.   The vegetation of this belt changes in the vicinity of Los Angeles Bay, as previously stated, more through the appearance of several additional

species than because of any difference in physiognomy or structure of the communities.   Here may be seen very open areas of *Larrea* accompanied by *Jatropha spathulata, J. cinerea, Cercidium micro-phyllum, Fouquieria peninsularis, Olneya tesota, Bursera micro-phylla* and *Opuntia cholla.*   There are few large cacti, no platyo-puntias, no semi-succulents, no leaf succulents and very few per-ennial grasses.

Geological evidence indicates that the separation of Baja Cali-fornia from the mainland is very ancient, even if the width of the Gulf has not been continually as great as it is to-day.   In recent time the Gulf has formed an effective barrier to plant migration and has virtually given insular conditions to the lowest third of the peninsula.   The vegetation and flora on the two sides of the Gulf are very similar above Guardian Angel and Tiburon Islands, but show greater dissimilarity south of that region.   The flora of Baja California has long been noted for its strong endemism, although recent exploration has found a number of its peculiar species on the mainland.   As previously stated, there are a number of cases in which geminate species, or members of closely related genera, fill the same place in the composition of the vegetation on the peninsula and in Sonora.

The Chihuahuan Desert.   The North American Desert lacks very little of being a continuous area from the state of Washington to the state of Puebla.   It is broken, however, by the high plains which straddle the continental divide in southwestern New Mexico and by the Sierra Madre range in western Chihuahua.   The entire drainage of the Chihuahuan Desert is into the Atlantic or into en-closed basins.   In southeastern Arizona and southwestern New Mexico there are many areas which appear at the present time to be almost as desert as others that are included in the desert bounda-ries of the accompanying map.   There are scattered pure stands of *Larrea,* and there are three of the common plants of the Chihua-huan Desert (*Acacia vernicosa, Mortonia scabrella, Flouresia cernua*) which occur locally.   On these plains the elevation is near 4000 ft. and scores of the characteristic species of the deserts are absent.   Also, there is trustworthy evidence that the entire ele-vated region between Benson, Arizona, and Deming, New Mexico, formerly supported a fairly heavy stand of perennial grasses, and that *Larrea* and *Prosopis* were much less abundant under the

original conditions. It therefore seems best to regard this region as part of the Desert-Grassland Transition region rather than part of the desert.

The Chihuahuan Desert includes parts of New Mexico and western Texas adjacent to the Rio Grande, the lower valley of the Pecos, the eastern half of Chihuahua, the western half of Coahuila, and parts of Durango, Zacatecas, Nuevo Leon and San Luis Potosi. A small percentage of the area along the Rio Grande is below 3000 ft. in elevation, nearly half of the Chihuahuan Desert is over 4000 ft. and some of the highest parts are over 6000 ft. Desert plants may frequently be found at 7000 to 8500 ft. Vast plains and immense undrained basins alternate with mountain ranges rising from 2000 to 5000 ft. above the plains, or with intricate groups of limestone hills. Hard surfaces are prevalent and the only extensive sandy area is immediately south and south-west of Ciudad Juarez, in Chihuahua. Limestone is far more abundant than in any of the other deserts. Outcrops of granite are uncommon at elevations occupied by desert. Deposits of gypsum and soils heavily impregnated with it are of wide-spread occurrence. A number of streams with heavy summer discharge drain the east face of the Sierra Madre Occidental in Chihuahua and Durango and end in extensive alkaline flats.

In the Chihuahuan Desert the rainfall ranges from annual averages of 3 in. in the broad undrained basins of Coahuila to amounts as high as 12 to 16 in. at more elevated stations near the western and southern edges of the desert. At nearly all of the stations the summer precipitation of June to September inclusive is from 65% to 80% of the annual total. There is light precipitation from October to the end of the year. The period from January to May is very dry throughout the area. Moderate frosts are universal at desert levels or may be severe above 5500 ft. The summer daytime temperatures are from 10 to 20° F. lower than those of the Sonoran Desert, except at low elevations along the Rio Grande. The rainfall and temperature conditions result in a single growing season, which is both longer and more certain than the summer season of plant activity in other sections of the North American Desert. There is no display of winter herbs, no winter germination, and no winter flowering, although there are many plants which remain wholly or partly in leaf during the cold months.

In the number of life-forms prominently represented in its vege-

tation the Chihuahuan Desert occupies an intermediate position between the poverty of the Great Basin and the richness of the Sonoran Desert. Shrubs and semi-shrubs are the predominant plants. Trees are small, few and confined to the margins of stream-ways or to rocky slopes. Stem succulents are abundant but not conspicuous. Two species of arborescent opuntias are wide spread and locally very abundant, platyopuntias are locally common, and two large barrel cacti are more conspicuous than abundant. The smaller stem succulents, from 6 to 12 in. high, are very abundant, and it is not uncommon to find 8 or 10 species in an area of 100 sq. ft. The columnar type of cactus barely enters the southern end of this desert. The semi-succulents—*Yucca, Nolina* and *Dasylirion*—are the most conspicuous of the larger plants and prove to be accurate indicators of habitat conditions. The leaf succulents—*Agave* and *Hechtia*—are of wide occurrence and particularly abundant. *Fouquieria* occurs throughout the Chihuahuan Desert. In certain localities grasses are abundant in the upper margin of the desert, while in other places they fail to appear until an elevation is reached which is above the vertical limit of the desert shrubs and cacti.

Lists of the 12 most common and characteristic plants of the Sonoran and Chihuahuan Deserts have only three species in common (*Larrea, Fouquieria, Prosopis*). This gives a vivid, but perhaps slightly exaggerated, idea of the floristic distinctness of the two areas. The Leguminosae and Compositae are both richly represented in each of the areas but the former family appears to be better represented in the Sonoran Desert and the latter in the Chihuahuan.

An important biological line of demarcation in the Chihuahuan Desert is the series of mountains running east and west near the southern boundary of Coahuila, including the Sierra de Parras and Sierra Jimulco. There are several commonly represented communities of plants which range with little change from New Mexico and western Texas to this transverse series of mountains. An almost equal difference in vegetation and flora exists between the eastern and western sides of the area, both north and south of the transverse ranges.

The forested and grassy highlands of western Chihuahua give source to three rivers which flow north into the extreme northern

part of the state and end in large lake beds. Surrounding these beds and largely due to them is a sandy area about 100 miles square. Only a small part of this area has reached an advanced stage of stabilization and much of it is occupied by active dunes from 50 to 300 ft. in height. The vegetation is sparse and irregular, varying with the stages of development of the surface. The area has been briefly described by MacDougal and Coville (1903) and by Shreve (1939). The characteristic plants of the dune area are *Prosopis chilensis, Artemisia filifolia, Dalea scoparia, Hymenoclea monogyra, Yucca elata* and *Poliomintha incana.*

From the dune region east to the valley of the Rio Conchos the vegetation of Chihuahua is a uniform and monotonous stand of *Larrea* or of *Larrea, Flouresia cernua* and *Acacia vernicosa* with broad low buried trees of *Prosopis* on the low ground. The leafless green-stemmed shrub *Koeberlinia spinosa* is very abundant with *Prosopis* in this region, being widespread but uncommon in the Sonoran Desert and in other parts of the Chihuahuan Desert. The secondary shrubs which accompany *Larrea* and *Flourensia* rarely form as much as 10% of the stand, quccas are not common and cacti are frequent but not conspicuously abundant.

East of the Rio Conchos a more diversified region extends to the limit of the desert in northern Coahuila. An increasing percentage of the surface is occupied by hills and small mountain ranges, in which granite, sandstone and limestone are represented, and the general elevation of the intermont plains reaches 5000 ft. In this territory the desert merges very irregularly into grassland or into evergreen oak shrubbery.

The occurrence of perennial grasses must nowhere be taken as an indication that the limits of the desert have been passed, since they are at least an infrequent element in its composition in the dryest parts of the Sonoran and Mojave Deserts. When, however, the grasses form a turf which covers 50% or more of the surface, when the shrubs and cacti are very widely spaced, and some of the desert dominants have disappeared, it is an indication that the transition to grassland has been entered. In northern Chihuahua there are a number of extensive level plains which are heavily and exclusively covered with grasses, almost solely *Hilaria mutica.* These "llanos" occupy the deep fine soil of the bottoms of undrained basins into which there has not been sufficient drainage to result in

the development of a central alkaline dry lake. Although they are grass-clad the llanos are not to be regarded as part of the grassland formation but rather as a local desert association. This is confirmed by the absence or scarcity of the grass species which are dominant in the true grassland, by the similar occurrence of *Hilaria* llanos at very low elevations in Arizona and Sonora, and by the presence of typical *Larrea, Flourensia* and *Acacia* desert on the bajadas surrounding and overlooking them.

South of the City of Chihuahua the drainage of the eastern slopes of the Sierra Madre as far as the borders of Durango finds an outlet to the Rio Grande through the Rio Conchos. All of the eastward drainage in Durango and Zacatecas finds its way into enclosed basins. The smaller basins of northern Chihuahua containing the llanos receive only local drainage. The larger alkaline basins of Chihuahua and Coahuila receive mountain drainage. Almost every map of Mexico indicates that the northern plateau is occupied by a single vast central basin designated the "Bolson de Mapimi," the exact location of which varies from map to map. There is no single bolson, or enclosed basin, of such extent, but rather a large area in which there occur many bolsons of various sizes and of the two types that have been indicated.

The greatest extent of deposition on the plateau has been in southern Coahuila around the Laguna Mayran which receives the drainage of the important Rio Nazas. This is the well known "Laguna District," in which there has been one of the most extensive developments of irrigated farming land in Mexico. Hundreds of square miles in the center of this basin were originally covered with an irregularly distributed wilderness of *Prosopis, Atriplex, Suaeda, Allenrolfea* and other halophytic plants. Such situations, indeed, are more nearly alike throughout the North American Desert than any other type of environment. There is little change in elevation northward and eastward from Laguna Mayran and much of the southern end of Coahuila is without permanent or extended drainageways. A ground water supply adequate for *Prosopis* is confined to the vicinity of the sinks and the western edge of the basin. The floor of the basin is level for long distances and in some districts at least one fourth of the area is devoid of plants. Elsewhere there is an extremely open cover of *Larrea, Lycium, Atriplex* and *Acacia*. In some districts the soil contains enough

small stones to have developed an open desert pavement. It frequently happens that a new and slightly lower level is established by erosion, resulting in a marked difference of vegetation on the two levels. On the lower level are the plants that have just been mentioned while on the upper slightly stony level there is a heavier stand of *Larrea, Fouquieria, Agave lechuguilla* and *A. falcata.*

For about 125 mi. north northwest from Saltillo the Sierra Madre is low and dispersed and there is a large region around Monclova which combines the characteristics of the desert with those of the arid bushland of Tamaulipas. A short distance northwest of Monclova the mountains again become more massive and the boundary of the desert more distinct. West of this locality, and half way across to the western edge of the plateau, the Chihuahuan Desert presents its most arid and austere aspect. The plant cover is scanty, open and low, devoid of small trees, and very poor in succulents and semi-succulents. The slopes of the mountains are so barren as to appear devoid of plants when seen at a short distance. The soil is highly alkaline, and the rainfall of the only two available stations varies annually from 3 to 6 in.

The arid central district of Coahuila has few large perennials which give character to its vegetation. In certain localities *Larrea* is outnumbered by the smaller gray *Sericodes Greggii*, of the same family. Gentle slopes are occasionally covered with thickets of the low, gray, stout stemmed *Grusonia Bradtiana*. Among the smaller perennials and the herbs, however, are a large number of plants with striking pecularities of habit or structure, including the dwarf *Prosopis cinerascens, Salazaria mexicana, Stillingia Torreyana* and local representatives of *Petalonyx, Selinocarpus, Nama, Cyphomeris, Anulocaulis, Heliotropium* and *Gaillardia.*

The most distinctive type of vegetation in northern Coahuila is that which covers the rolling, hilly or very irregular limestone areas, the total extent of which is very great. The shrubbery of the limestone areas is open and low except above 5000 ft. The variety of life-forms is much greater than elsewhere, with the abundance of *Fouquieria* and *Agave,* the frequency of *Yucca* and *Hechtia,* the wealth of small cacti and the presence of *Jatropha spathulata* and the leafless succulent *Euphorbia antisyphilitica.* Perennial grasses are scarce, and throughout the Chihuahuan Desert it may be noted that the transition from Desert to grassland takes

place at a higher elevation on limestone than on other substrata, the highest observed case being at 8000 ft. in northern Zacatecas.

Gypsum deposits are widely distributed from Texas to San Luis Potosi but their aggregate area is far less than that of limestone. They are usually betrayed by the very white surface, the absence of shrubs and the low uniform cover of distinctive plants. Johnston has recently (1941) listed 28 species and varieties which appear to be obligate gypsophiles. The commonest ones are *Muhlenbergia villiflora, Sartwellia mexicana, Dicranocarpus parviflorus, Nama Purpusii* and *Dalea Filiciformis*.

South of the mountains which have been mentioned as crossing the southern end of the Chihuahuan Desert is a series of high peaks and ridges in the vicinity of Concepcion del Oro and Mazapil, Zacatecas. High rolling plains extend southwest from these peaks to the city of Zacatecas. The height of land then veers toward the southeast, becoming the continental divide and extending beyond the Valley of Mexico. The southwestern extremity of the Chihuahuan Desert occupies the lower drainage basin of the Rio Aguanaval, in Durango and Zacatecas. On the west and south this area is definitely bounded by grassland and forest. East of the high rolling plains of northern Zacatecas is the southeastern extremity of the Chihuahuan Desert, occupying parts of Nuevo Leon and San Luis Potosi. On the east this extension is bounded by the Sierra Madre Oriental and on the southeast merges rather rapidly into a different type of desert which extends discontinuously far south through Hidalgo and Puebla.

The valley of the Aguanaval has many resemblances to northern Coahuila in its landscapes and its flora but not in the physiognomy of its vegetation. On account of the elevation, which is from 4000 to 7000 ft., and the rainfall, which is from 8 in. to 15 in., the vegetation is heavier than in northern Coahuila. *Larrea* reaches a height of 5 to 6 ft., *Prosopis* is common as a small tree, and 25 to 30% of the vegetation is made up of *Acacia, Celtis, Leucophyllum, Cordia, Condalia, Lycium, Lippia* and members of other shrubby genera. In suitable situations *Yucca australis* is large and abundant and *Opuntia imbricata* and *O. Kleiniae* are common and ubiquitous.

The southeastern arm of the desert is much more arid than the southwestern. The great open expanse of northern San Luis

Potosi, known as the Valle del Salado, has an annual rainfall of about 4 in.   There is no outlet for the drainage and the soil is everywhere very alkaline or else has a high content of gypsum. Immense tracts are covered with *Larrea* from 2 to 3 ft. high, either nearly pure or competing with *Flourensia cernua* in places where the soil moisture is sometimes higher.   Very open forests of *Prosopis* occupy favorable sites, with a heavy ground cover of *Atriplex*.

It is true here, as it is throughout the desert, that the most widely distributed and drought-resistant plants are the ones which dominate the intermont plains.   In every part of the desert it is the plants of the upper bajadas and hills which give the vegetation of the region its distinctive character and include in their number the species which are of special interest on account of their structural modifications, relationships or geographical range.   Also, on approaching the edge of the desert or of one of its subdivisions, it is the upland rather than the valley plants which give the first suggestion of impending change.   In southern Nuevo Leon and central San Luis Potosi the hills and rocky bajadas suggest the density of the Tamaulipan arid bushland and the diversity of life-forms of the Sonoran Desert, at the same time that a new floristic element finds here its northern limit.   In the increased density of the shrubbery a larger number of species is involved than exists elsewhere in the desert, including notably several abundant species of *Dalea*.   The larger plants include small trees of *Prosopis, Acacia, Bursera, Cassia, Citharexylum* and *Karwinskia*.   The leafless green-stemmed trees are represented by *Acanthothamnus aphyllus*. Columnar cacti again appear in *Myrtillocactus* and *Lophocereus,* at the same time that tall platyopuntias take a prominent place in the landscape.   Several species of *Yucca* are tall and abundant and locally the slopes are covered with *Dasylirion longissimum* which has a short stout trunk from which at least a thousand linear leaves curve gracefully to a length of 6 ft.   The small desert agaves are now rare but tall massive ones are abundant.   In many situations the twigs of the trees are covered with the epiphytic *Tillandsia recurvata*.   With all of these varied elements the structure and social organization of the communities are profoundly altered.   Most noticeable is the occurrence of a relatively heavy growth of herbaceous plants in the shade of the larger ones and the scarcity of suit-

able spots for the germination of the wide-spread desert perennials which are here so conspicuously absent.

The Arid Regions of Southern Mexico

South of the Chihuahuan Desert there are discontinuous areas in which the vegetation differs widely from that of the four subdivisions that have been described but nevertheless exhibits the essential characteristics of the less extreme types of desert. These areas are rich and diversified, with a large number of life-forms and many plants of distinctive habit, structure and behavior. The stature and density are very irregular but exceed those of the northern sections of the desert. The minimal area has not been determined but is undoubtedly greater than elsewhere in the desert. The flora is much larger than in any of the regions that have been described.

The southern desert areas are closely related to the arid bushland and to the thorn forest, and merge gradually into these formations. Desert plants and ecological features of the desert recur again and again in the rugged terrain of southern Mexico, although often in areas which can not be regarded as desert.

The time is not ripe for an adequate discussion of the arid regions of southern Mexico and their relation to the northern areas of more pronounced desert. There has been little ecological investigation of these regions, and knowledge of their flora is far from approaching completeness. Miss Bravo has described the Valley of Actopan (1936) and the Valley of Mesquital (1937), in Hidalgo, and MacDougal has briefly described the district of Tehuacan, in Puebla.

It has been noted that in northern Mexico the broad valley floors have the most simple and drought-resistant vegetation. In southern Mexico the valley floors are again the most arid habitat and the one in which the vegetation most nearly resembles that in the north. Throughout Hidalgo, Puebla and adjacent states a very slight amelioration of the soil and soil moisture conditions suffices to support arid bushland, thorn forest or other types of xeric or hemixeric vegetation. In and around the arid valleys are hills, canyons, barrancas and narrow flood plains on which a more mesic vegetation is dominant.

All of the life-forms found in the Sonoran and Chihuahuan

Deserts are represented in the southern desert areas. Almost all of the features of form, habit, structure and behavior that are the distinctive characteristics of desert plants are found in the southern areas, either in their initial or their final phases of development. Also many of the genera which have contributed one or two highly specialized forms to the pronouncedly desert areas are represented in the south by a larger number of species. In the members of these genera may be detected the structural features which have undergone further development in certain species, making life possible for them in the very arid regions.

The small size of leaves or leaflets is a very common feature of the plants of arid southern Mexico. The assumption of photosynthetic work by the stem is common, although not often accompanied by total loss of the leaf. Plants with succulent leaves are even more abundant in the south than in the north. Semi-succulent plants are as abundant in species in the south but not so numerous in the vegetation. The stem-succulent cacti are perhaps even more abundantly represented in both species and individuals in the south than they are in the Sonoran and Chihuahuan Deserts.

Present knowledge of the distribution of the life-forms and genera of American desert plants suggests the existence of a small group of old races, probably of desert origin, and of a larger group made up of migrants from the arid and semi-arid regions of southern Mexico. The regions in which the presumably old element is strongest are the northern part of the Sonoran Desert and the northern central basins of the Chihuahuan Desert. The newer element is best represented in the southern parts of the Sonoran and Chihuahuan Deserts.

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TAXONOMY AND PHYLOGENY

W. B. TURRILL

Royal Botanic Gardens, Kew, Surrey, England

PART I

CONTENTS

INTRODUCTION

Scope of the Paper

The wide title given to this review may be misleading unless an explanation of deliberate limitations be given. Firstly, we are concerned mainly with the results of botanical studies. The conclusions or opinions of zoologists are quoted only when they supplement or contrast with those of botanists, so that, while an attempt has been made to give a broad representation of views published in books and papers by botanists, a very meagre selection of those by zoologists is quoted. This is not done without realization that the problems to be discussed are essentially biological rather than either botanical or zoological, but the writer is a botanist possessing only a limited acquaintance with special zoological problems and literature. Moreover, this paper was prepared for and is published in a botanical periodical. Secondly, it is the general problems of taxonomy and of phylogeny and the actual, supposed and possible relationships between them which are discussed. Published systems of taxonomy and schemes of phylogeny are not reviewed in detail, although reference is made to a fair selection of them. Thirdly, except in the final considerations, criticisms by the writer and his personal conclusions have been reduced to a minimum, though attention has been drawn to fallacies where it has seemed particularly desirable to do this. On the positive side, em-

phasis has been given to such subjects, whether special or more general, as seem to be in need of further investigation, or the more critical consideration of which might well lead to marked improvement in taxonomy or advance in knowledge of phylogeny or both.

Derivations and Definitions of Terms

In Murray (317) a considerable number of senses are given for the word *class* (Latin *classis*). For our present purpose the most important is: "A number of individuals (persons or things) possessing common attributes, and grouped together under a general or 'class' name; a kind, sort, division. (Now the leading sense.)" It should also be noted that class is one of the higher of the hierarchical divisions in many biological taxonomic systems. In the same work (317) *classification* is given two senses: "1. The action of classifying or arranging in classes, according to common characteristics or affinities; assignment to the proper class. 2. The result of classifying; a systematic distribution, allocation, or arrangement, in a class or classes; esp. of things which form the subject-matter of a science or of a methodic inquiry".

The word *affinity* is derived from the Latin *affinis*. Lewis and Short (266) give the following: "I. that is neighbouring or a neighbour to one; contiguous, bordering on, adjacent; also near by family relationship, allied or related to by marriage (opp. consanguinei). II. partaking, taking part in, privy to, sharing, associated with". The earliest use in English dates back at least to 1303 and indicates relationship by marriage as opposed to consanguinity. Murray (317), for the natural history sense of the word, gives: "Structural resemblance between different animals, plants, or minerals, suggesting modifications on one primary type, or (in the case of the two former) gradual differentiation from a common stock". He gives for this as the earliest quotation: Sullivan, View of Nature 1. 458: 1799: "Thus we shall find that antimony has an affinity with tin".

The noun *taxonomy* was invented by A. P. de Candolle (128) in 1813. He distinguished *glossologie* as "la connaissance des termes par lesquels on désigne les organes des plantes et leurs diverses modifications"; *taxonomie* as "la théorie des classifications appliquée au régne végétal"; and *phytographie* as "l'art de décrire les plantes de la mannière la plus utile aux progrès de la science".

In a footnote he explains that the word taxonomie is derived from the Greek ταξεις, order, and νόμος, law. De Candolle estimated that in 1813 some 30,000 species of plants were known, and asked how we can keep clearly before our eyes this immense study. He concludes: "Ce service éminent, nous ne pouvons l'attendre que d'une méthode telle, qu'après avoir divisé successivement en plusieurs groupes ces nombreux individus du règne végétal, nous arrivions per une marche sûre à connaitre celui qui nous intéresse: c'est cette partie de l'étude des végétaux, que je désigne sous le nom de *Taxonomie botanique*".

Diels (131) also clearly separates *phytography* from *systematics*. The latter was at first directed to the logical arrangement of the abundance of plant kinds. To this end, the characters of plants, as supplied by phytography, were compared and established in order to decide which gave the best required arrangement. "Dabei entwickelte sich immer stärker das Bemühen, die 'natürliche Verwandtschaft' der Pflanzen zu ermitteln, wie sie sich durch die Gesamtheit der Merkmale kundgibt. . . . Und als die Deszendenzlehre sich durchsetzte, musste die Systematik ihr Ziel darin sehen, die Beziehungen der Pflanzen als echte genetischen Verwandtschaften nachzuweisen".

The word taxonomy was, we have seen, introduced by a botanist, and, one suspects, it is even now more used in botanical than in zoological literature. It is interesting also to note that it was deliberately applied to the theory of plant classification and not as a general term to cover all the activities of the systematist. The connotation was, however, rather quickly widened. Thus, the Encyclopaedia Britannica, in the seventh edition, 1832, says: "Taxonomy is that branch of botany which has for its object the combination of all our observations on plants, so as to form a system or classification". Asa Gray (169), in his Glossary, defines taxonomy as "Relating to classification and its rules", but in one place in the text he limits the term to the principles of classification and in another extends it to the study of classification. Murray (320) gives the following sense (or more accurately senses) for taxonomy: "Classification, esp. in relation to its general laws or principles; that department of science, or of a particular science or subject, which consists in or relates to classification". Jackson (242) defines taxonomy, with *taxology* as an alternative, simply as classification.

Hitchcock (221) says: "Taxonomy is the science of classification, more especially as applied in biology". On the other hand, many biologists would limit the term taxonomy to the classification of plants and animals into families, genera, species, *etc.*, designated by Latin names and associated with formal descriptions based on the greatest possible number of resemblances and differences—thus excluding, at least, special classifications based on one kind of data (ecological, genetical, *etc.*) only. The tendency of the newer school of taxonomists to expand the methods and content of taxonomy has been discussed by Diels (131), Stojanoff (412), Lam (253), and Turrill (456).

The term *phylogeny* was invented by Haeckel in 1866 (181) and is derived from the Greek φῦλον, race (phylum), and -γενεια, birth or origin. Crow (115) translates Haeckel's definition of *Phylogenie* as follows: "the science of the form-changes which the phyla or organic races pass through during the whole period of their individual existence". The process of development itself was later termed *phylogenesis* by Haeckel who added the following, as translated by Crow: "The science which has for its object the empirical knowledge of these historical facts, and the philosophical perception of their causes, we call race-history or phylogeny. From the nature of the facts the latter belongs to the historical natural sciences, for it investigates events the direct observation of which is, in by far the greater part, impossible". Murray (319) gives the following senses: "1. The genesis and evolution of the phylum, tribe, or species; ancestral or racial evolution of an animal or plant type (as distinguished from *ontogenesis,* the evolution of the individual). 2. The history or science of evolution or genealogical development in the phylum, tribe or species; the race history of an animal or vegetable type; tribal history. [For this sense, Huxley, Anat. Inv. Anim. Intr. 41: 1877, is quoted: 'A special branch of biological speculation termed phylogeny']. 3. A pedigree or genealogical table showing the racial evolution of a type of organism".

A group of British biologists in 1938 agreed tentatively to the following definition of phylogeny: "the process of the production or origin (or origins) of a group (or groups) of organisms and of the evolutionary development of the group or groups" (unpublished). Gilmour (163) suggests that phylogeny may be defined as "the history of the origin and development of taxonomic groups".

The terms *monophyletic* and *polyphyletic* are commonly used more or less vaguely to indicate origin of a group from one or from more than one group, respectively. Sometimes they are used in a taxonomic sense, sometimes in a genealogical sense, especially with reference to a common ancestor. Thus, a genus arising from another would be monophyletic taxonomically, even if its constituent species originated from more than one species of one ancestral genus, but it would be polyphyletic genealogically. If the terms be used taxonomically, it must be decided which relationship between the ranks of the originating and resulting groups constitutes monophyly and polyphyly; if they be used genealogically, any group which has been formed by distinct lineages must be called polyphyletic. Smith (406) also notes that: "To some extent the argument is one of the meaning of terms. If the ancestors of a genus are known and if these are regarded as distinct genera, we have a polyphyletic genus; if the ancestral complex is taken as one genus, the present-day resultant is monophyletic. We are back again at the problem of the status of genera and the easy production of intergeneric hybrids if subdivision is carried far enough".

Clements (107) defines *polygenesis* as: "the origin of a form at two or more distinct places or times". He then distinguishes within polygenesis, origin in place from origin in time, as follows: "A form that arises in two or more places is called *polytopic,* one originating at different times, *polychronic.* Contrasted with these are *monotopic* forms which originated but once".

The terms *analogue* and *homologue* (analogy and homology and other derivatives) have been widely used in other than biological subjects, but were precisely defined for biological use by Owen in 1843 (331, 332) as follows: "Analogue—A part or organ in one animal which has the same function as another part or organ in a different animal. Homologue—The same organ in different animals under every variety of form and function."

Ray Lankester (263) introduced the word *homoplasis* or *homoplasy.* The following quotation gives the use he intended should be made of the term: "When identical or nearly similar forces or environments, act on two or more parts of an organism which are exactly or nearly alike, the resulting modifications of the various parts will be exactly or nearly alike. Further, if, instead of similar parts in the same organism, we suppose the same forces to act on

parts in two organisms, which parts are exactly or nearly alike and sometimes homogenetic, the resulting correspondence called forth in the several parts in the two organisms will be nearly or exactly alike. I propose to call this kind of agreement homoplasis or homoplasy". Interesting discussions on and examples of homology, analogy, and homoplasy (parallelism, convergence) have been published (17, 54, 136, 158, 165, 261, 262, 309, 399), and Gates (158) points out that the concept of parallel mutations was originally based on an example in *Oenothera*.

HISTORICAL DEVELOPMENT OF PLANT CLASSIFICATION
Pre-Linnaean Systems

Throughout the realm of nature, more or less exact repetition of phenomena in space and time imposes on human mental activities, the necessity of grouping into classes as a means of reducing the number of items to be considered in any given connection. Since it is doubtful that any two phenomena can ever be exactly the same, intrinsically and in their external relationships, the degree of resemblance which determines class identity has to be fixed to this extent arbitrarily. Even the phenomenal units to be classified have to a varying extent to be chosen. While individuality is perhaps more clearly distinguishable in biology than in other branches of natural knowledge, the difficulties of defining an individual is well known to botanists and zoologists, and usually involves or should involve a definition for each piece of published work concerned with individuals (232, 93, 314, 64, 135).

That prehistoric man grouped together similar phenomena is evident from what we know of his activities and mental outlook through modern archaeological research. Symbolism, for example, in some of its primitive forms rests on classification. The origin of language, especially the invention of nouns which may well have been the first words for communication of ideas, implies classification, as does also the, presumably later, making of records, whether mnemonic, pictorial, ideographic or phonetic, which terminated in modern alphabets. For the classifying and naming of organisms we have the biblical statement (Genesis, 2, 19–20) that Adam gave names at least to "every beast of the field, and fowl of the air", the context clearly implying group names. That classification of animals and plants into kinds was primarily concerned with those ani-

mals useful or harmful to man, may be accepted both on general grounds and on a reasonable interpretation of available relevant archaeological and ethnological evidence.   An excellent and concise summary of what is known, through archaeological research up to 1927, regarding the first uses of plants by man, is given by Peake (334) with numerous bibliographical references.   Bartlett (32) shows how instructive is a study of the lore of plants, as traced in the records of Greece, Egypt, Palestine, Italy, China, the East Indies, Mexico, *etc*.   He says: "The concept of the genus must be as old as folk science itself.   Two processes must have been operative in ancient times just as they are to-day.   (1) With enlarging experience, people make finer distinctions, and need different names for newly distinguished entities which have previously been called by the same original name.   The original name becomes generic in its application; variously qualified it provides the basis for specific names.   Thus genera are set up by analysis.   (2) As the language becomes cumbersomely rich in separate names for closely similar things, there is a tendency toward grouping or classification under the same name on the basis of newly perceived similarities.   Thus genera are set up by synthesis".   Later, he notes that: "The tendency to group plants into named genera, so generally characteristic of human thought and language, reflects the fact that there are not enough different words in the living, current vocabulary of any language to supply each closely similar plant with a basically distinctive name".

Theophrastus (424) used a classification of the vegetable kingdom into trees, shrubs, under-shrubs and herbs.   He suggested, however, other bases of classification, saying: "one must not make a too precise definition; we should make our definitions typical.   For we must make our distinctions too on the same principle, as those between wild and cultivated plants, fruit-bearing and fruitless, flowering and flowerless, evergreen and deciduous".   He remarked that some of the differences he mentioned are: "differences of natural character, as it were", and he very carefully enumerated differences in habit, roots, leaves, seeds, flowers, fruits, *etc.*, and stressed also the importance of locality, indeed, of what we should now term ecology in a broad sense.   In the Introduction to his translation of "Enquiry into Plants," from which translation the above quotations are made, Hort informs us that Theophrastus "was born in

370 B.C. at Eresos in Lesbos; at an early age he went to Athens
and there became a pupil of Plato. It may be surmised that it was
from him that he first learnt the importance of that principle of
classification which runs through all his extant works . . . and
which is ordinarily considered as characteristic of the teaching of
his second master, Aristotle. But in Plato's own later specula-
tions, classification had a very important place, since it was by
grouping things in their 'natural kinds' that, according to his later
metaphysics, men were to arrive at an adumbration of the 'ideal
forms' of which these kinds are the phenomenal counterpart, and
which constitute the world of reality. Whether Theophrastus
gathered the principle of classification from Plato or from his fel-
low-pupil Aristotle, it appears in his hands to have been for the
first time systematically applied to the vegetable world. Through-
out his botanical works the constant implied question is 'What is
its difference?' 'What is its essential nature?', *viz.* what are the
characteristic features in virtue of which a plant may be distin-
guished from other plants, and which make up its own 'nature' or
essential character?" Theophrastus died about 285 B.C.

Arber (20) notes that: "In the *De materia medica* of Dioscor-
ides, there is little attempt at arrangement, even in those versions
which are not merely alphabetical. Book III, for instance, gives
an account of roots, juices, herbs and seeds used for food or medi-
cine, and the other books are defined with equal vagueness. Here
and there, however, a slight feeling for kinship emerges; a number
of umbellifers, for example, are enumerated in succession".

Arber (20) may again be quoted as an authority on herbals, re-
garding which she says: "we find that the earliest show scarcely
any trace of a natural grouping, the plants being, as a rule, arranged
alphabetically. This is the case, for instance, in the Latin and the
German "Herbarium", the "Ortus sanitatis", and their derivatives,
and even in the great herbal of Leonhart Fuchs in the sixteenth cen-
tury. In Bock's herbal, on the other hand, the plants are grouped
as herbs, shrubs, and trees, according to the classical scheme. The
author evidently made some effort, within these classes, to arrange
them according to their relationships", as is shown by a quotation
from the preface of his Kreuter Büch (1551).

More or less definite attempts to find natural groupings, relation-
ships or affinities amongst the various known kinds of plants, are

enumerated by various authors (20, 368, 396), and these resulted first in a conception of genus and species, a grouping together of similar kinds, which gradually became clearer. "We owe to Konrad Gesner the first formulation of the idea that genera should be denoted by substantive names", but "it was left to Fabius Columna to publish definite views as to the nature of genera" (20). Where there is, in the earlier herbals, any sustained attempt at a general classification for all known kinds of plants, it is largely on a basis of uses to man, though, with ups and downs, structure of the plants themselves became more and more important as a basis of classification, that is, the first glimmerings of a natural system begin to appear. Arber (20) says: "There seems little doubt that de l'Obel [1538–1616] made a more conscious effort than any of his predecessors to arrive at a natural classification, and that he felt that such a classification would reveal an underlying unity in all forms of life", an arrangement of plants "according to their kind and their mutual relationship". Gaspar Bauhin [1560–1624] was, on the whole, "markedly successful in recognizing affinities within small cycles, but he broke down on the broader question of relationship between the groups of genera so constituted. . . . Like de l'Obel, Bauhin seems to have believed in the general principle of a progression from simpler to more highly progressive forms".

The works of Robert Morison [1620–1683] are considerable if not numerous and have been concisely reviewed by Vines and Druce (471). In the history of plant classification, he is particularly prominent as the author of a folio treatise on the Umbelliferae [1672], the first systematic monograph of a family of plants in any approximation to the modern sense. He did not live to complete his great work, "Plantarum Historiae Universalis Oxoniensis". In the sections and divisions of plants which he established, Morison's system sufficed to group together many plants which are brought near one another in modern natural systems. Vines and Druce were able to attach modern equivalents, in the form of family or generic names, to some of these groups. Morison classified the Umbelliferae according to fruit (seed) characters and largely utilized them in his general classification of herbs. The degree of natural grouping found in Morison's *magnum opus* is probably more accidental than intentional, since the use of type or key characters is undoubtedly the underlying intention of the system.

Ray [1627–1705] classified all the plants made known by his predecessors and contemporaries, according to Singer (396) about 18,600 in number, into 125 sections of which a number bear a definite resemblance to modern families (352). Ray, in 1705, introduced the terms "monocotyledons" and "dicotyledons", but still retained as a main division the distinction between herbs and woody plants.

Linnaeus

The contributions of Linnaeus to taxonomy are so well known and have been the subject of so much research that only a brief reference will be made to them here. By his clear division of genera and species and his fixation of the binomial system, Linnaeus established methods which are still followed by all the orthodox in the general taxonomy of animals and plants. His system of classification, based on the androecium and gynoecium for flowering plants, is usually quoted as the classic example of an "artificial" system. On the other hand, as Sachs (368) shows, Linnaeus was the first to say clearly that there was a natural system of plants which could not be established by the use of predetermined marks, as had previously been attempted, and that even the rules for framing it were still undiscovered. In his "Fragments" [1738] he gave a list of 65 groups which he regarded provisionally as cycles of natural affinity which were "founded solely on a refined feeling for the relative resemblances and graduated differences that were observed in comparing plants with one another". Around this period, both before and after Linnaeus, the idea gained ground "that there is a common type lying at the foundation of each natural group" from which all its forms, though specifically distinct, can be derived, as the form of a crystal can be derived from one fundamental form. It must be remembmered that the dogma of the constancy of species was accepted as orthodox, not merely scientifically, though, as Ramsbottom (349) has clearly shown, Linnaeus's views underwent certain changes in this direction, and "from the first he regarded his Sexual System as one of convenience until the time when a Natural System could take its place, though admittedly he thought the time far distant".

Post-Linnaean and pre-Darwinian Systems

Adanson (1), in his Preface, which occupies more than half of the first volume (I. Partie), comments on the two kinds of methods

used by botanists: the natural and the artificial. He says: "La naturele est cele qui conserve, dans sa distribution toutes les Classes natureles, c.à.d. des Classes où il n'entre aucunes Plantes qui ne convienent entr'eles. C'est la nature qui prescrit ici à l'Auteur métodiste la marche qu'il doit suivre, ... La Métode artificiele est celle dont les Classes ne sont pas natureles, parce qu'eles reassemblent des Genres de Plantes très-éloignées, & qui n'ont pas le plus grand nombre des raports nécessaires pour les raprocher, quoiqu'ils convienent ensemble par la note ou les notes caractéristikes assignées à chake Classe". Again, he notes that the natural method "se régler sur la nature des êtres, qui consiste dans l'ensemble de leurs Parties & de leurs qualités". Even his families are based "sur toutes les parties". Adanson clearly accepts transmutation of species and further argues that species and other divisions of systematic biology have an existence, even if only relative to us. He emphasizes the importance for a natural system of "Caracteres de l'Ensemble". The idea of utilizing both the vegetative and reproductive parts of plants in classification is clearly shown in his "Tableau des 58 familles des plantes" with which the second volume (II. Partie) commences. Although influenced and to a certain extent guided by the work of pre-Linnean systematists and by the fragment and comments on a natural system by Linnaeus, there is no doubt that Adanson occupies an important place in the development of systematic botany and that he was one of the more important of the founders of the modern family system.

Taxonomists immediately succeeding Linnaeus, for the most part, used his sexual system and devoted their energies mainly to describing and naming the wealth of new species and genera which botanical exploration brought to them from all parts of the world. Following Adanson, the next very important step in the formation of a natural system was the publication of the system of Bernard de Jussieu [1699–1777] by his nephew and assistant Antoine Laurent de Jussieu [1748–1836], under the title "Genera plantarum secundum ordines naturales disposita" (244). In this system the two primary divisions, for flowering plants, of Monocotyledons and Dicotyledons, were adopted from Ray, but a great advance was the distribution, in a much clearer manner than was done by Adanson, of the known plants into natural orders, in part corresponding to the families of modern classifications. This

further division depended partly on the number and arrangement of petals in the flower and partly on the position of the stamens with reference to the ovary (hypogynous, perigynous, epigynous). The names were given from the name of a genus in every group and not from certain "marks". Such a mode of naming probably expresses the idea, which from that time forward largely prevailed in systematic botany, that there is a common type lying at the foundation of every natural group from which all its forms can be derived (396). Fifteen classes and 100 natural orders are distinguished. The latter include six classed as Acotyledones: Fungi, Algae, Hepaticae, Musci, Filices, and Naiades. The last is, on any modern basis, a hopeless mixture of cryptogams and phanerogams.

Ideas of a natural system are more crystallized by de Jussieu (244) than by earlier authors. After considering the characters of plants, organ by organ as then understood, he concludes that all characters should be studied and weighed or calculated according to their relative values, in the sense that a constant character should be considered of value equal to several variable characters. To quote from the excellent critical examination by F. de Bondarot, Desfontaines, and Delamarck: "Ensuite M. de Jussieu, rappellant les comparaisons faites de la disposition des végétaux avec une chaîne dont les chaînons sont représentés par des plantes, ou avec une carte géographique dans laquelle chaque être occupe un point fixe, cherche à prouver que, si les matériaux de cet ordre sont difficiles á rassembler, les principes qui lui servent de base sont faciles à reconnoître & à fixer. Ainsi, ayant observé que l'espèce est la collection des individus absolument semblables, il a ajouté que, pour suivre la marche de la Nature dans le rapprochement des espèces, il faut joindre celles qui se resemblent par le plus grand nombre de leurs caractères, & il prouve la vérité de ce principe par l'examen de plusieurs genres très naturels & généralement avoués". Groups are determined by resemblances in characters, especially constant characters, uniform within the group and derived from a study of the essential organs, *i.e.*, the flower and seed. Groups thus formed are the natural classes in that they can be arranged in a chain or map to indicate the Order of Nature. In passing, it is interesting to note that in the examination signed by Geoffroy, Jeanroy, and Hallé, after referring to Jussieu's application of his principles in his search for "la méthode naturelle", this sentence occurs: "Deux

routes doivent, selon lui, nous conduire à la découverte de cette méthode: l'une, par une espèce d'analyse, conduit des observations aux principes; l'autre, par une méthode synthétique, conduit des principes démontrés à l'établissement des divisions principales qui n'en sont plus que les conséquences".

The meaning attached to affinity is indicated by the following quotations from Jussieu's Introductio: "in unam speciem colligenda sunt vegetantia seu individua omnibus suis partibus simillima & continuatâ generationum serie semper conformia. . . . [Specierum affinium connexio generica] Quae autem in unam compellit speciem individua omninò parilia, eadem specierum similium adjunctionem aequo jure lex determinat. Nam pariter, nemine diffitente, *consociandae sunt species majori caracterum* numero conformes, ac ideò dimovendae quae pluribus differunt signis. Haec prior connexionum ratio primam exhibet affinitatum trutinam quâ examinantur species quaelibet in eumdem fasciculum congregandae". Again, under the marginal title "Affini tatum leges naturales", he says: "Placitum illud, observatione confirmatum & inter leges verè naturales reponendum, priori additur legi quâ *consociandae sunt plantae majori caracterum numero* affines; & ex his duabus congruenter expositis ac amplificatis integra pendet genuinae plantarum distributionis norma".

In view of certain modern conclusions, discussed later in this paper, on the structure and relationships of families and other taxonomic groups, the following paragraphs from Robert Brown are quoted here: "Jussaeanam methodum itaque sequntus sum, cujus ordines prelique verè naturales, licet eorum classica dispositio, concedente Auctore non minùs candido quàm docto, saepè artificialis, et quandoque, ut mihi videatur, principiis ambiguis innixa.

"Nec vero pro illâ aliam substituere tentavi, nec de ordinam serie admodum solicitus sui, ipsa natura enim, corpora organica reticulatìm potiùs quam catenatìm connectens, talem vix agnoverit".

Bather (33) states that the image of a net to represent the ordered complexity of nature was first employed by Donati in 1750. He further quotes Linnaeus, 1756,: "plants show affinities on all sides, like a district on a geographical map"; and Cuvier, 1828,: "cet immense réseau qui constitue la nature organisée", as having the same conception of affinities.

Lamarck [1744–1829] was much impressed during the earlier and mainly botanical part of his career by the series of affinities and "la chaine admirablement graduée" presented by a systematic study of the vegetable kingdom as a whole (see, especially, 255).   The phrases "un ordre naturel", "le véritable plan de la Nature", and such like are apparently not given any evolutionary or phylogenetic significance.   He arranges his plants "à la suite les unes des autres en raison de leur rapports les plus marqués".   He says: "il faudra commencer l'ordre par quelqu'un de ces individus, qui, à raison du mécanisme imperceptible de leurs organes essentiels, sont à notre égard comme les premières ébauches des productions végétales. Ainsi il faudra se déterminer pour un *agaric*".   Later (256) he definitely states that he considers Jussieu's system the most natural yet imagined.   He is, in all his general accounts of plant classification, very clear about the practical purpose of classification, for what we should now term the determination of specimens.   At the same time he emphasizes the necessity of a classification to give a bird's eye view of the wealth of plant forms, indeed "d'embrasser à la fois, par l'imagination, tous les êtres naturels observés ou connus" (257).   This is an interesting aspect of classification which would repay full philosophical and methodological consideration.   It is obvious that Jussieu's work made a very strong impression on Lamarck, and it might be worthwhile for a biological historian to investigate how far it helped Lamarck to formulate his evolutionary ideas.

Lamarck's "Philosophie Zoologique" was published in 1809 (new edition in 1830) and his "Histoire naturelle des animaux sans vertèbres" from 1815 to 1822.   It was in these works that his conclusions on evolution were fully formulated, though the first outline was published in 1801.   The controversies which still continue regarding Lamarck's particular theories concerning the direct action of the environment, use and disuse of parts, *etc.*, on heredity and evolution, have unduly overshadowed his very solid contributions to the progress of biology and have also led to the neglect of other important ideas which he put forward with clarity and precision.

The earliest traced published statement of Lamarck's, giving an evolutionary explanation of the natural order of plants, was published in 1825 (258) and reads: "Revenons à la formation d'un

ordre naturel des végétaux, c'est à dire d'une distribution des familles, des ordres et des classes naturelles qu'on distingue parmi eux, distribution conforme à la marche de la nature et au plan qu'elle a suivi en produisant les êtres qui les composent". With this should be read a later paragraph: "Cherchant à déterminer le véritable ordre de la nature, j'ai dû, comme elle, partir de ce qui est le plus simple, et me diriger graduellement vers ce qui est le plus composé; j'ai dû commencer par le végétal le plus imparfait, et continuer ma série de masses en m'élevant jusqu'au végétal le plus composé dans son organisation et dans ses parties, ou du moins me laisser entraîner par les caractères généraux employés à la conservation des rapports naturels, jusqu'au terme qui présente la place du végétal le plus composé, c'est à dire dont les organes divers sont en plus grand nombre. J'ai pu ne pas atteindre le but; mais j'ai la conviction qu'il faut avoir égard à ces principes fondamentaux pour y arriver".

A. P. de Candolle (128) noted that 30,000 species of plants were known in 1813, with another 10,000 present in collections but not yet edited. He clearly showed the need for classification as a practical guide "dans ce dédale effrayant". Classifications may be empirical, as an alphabetical arrangement or other kind independent of the nature of the objects classified, or rational, depending on the nature of the objects. Three sorts of the latter can be distinguished: *a*) Utilitarian or Practical, when the plants are studied "quant à leurs rapports avec un autre ordre de connaissances", as according to their uses, properties, countries, *etc.; b*) Artificial, when the aim is to name plants as easily as possible; and *c*) Natural, when the aim is to study the plants "soit en elles-mêmes, soit dans les rapports réels qu'elles ont entr'elles, et les classer de manière que celles qui sont les plus voisines dans l'ordre de la nature, soient aussi les plus rapprochées dans nos livres". The natural system for plants can be determined only by "l'ensemble de leurs ressemblances anatomiques" and by arranging plants "d'après l'ensemble de leurs organes essentiels". He notes that, while a linear arrangement is necessary for publication in books, the plant kingdom can not naturally be disposed in a simple series. Groups are more or less and very unequally distant one from another and do not form a continuous series. The outline of his system tabulates 145 natural families, including ten of cryptogams. It is extremely impor-

tant, as the basis of the later and much used system of Bentham and Hooker, the resemblances between the two systems being, indeed, extremely close. De Candolle's system is quite definitely founded on comparative and correlative morphology (essentially gross morphology) and has no background of evolutionary or phylogenetic theory.

Lindley's classification (270) is based on the principle that "that which really determines affinity is correspondence in structure. It may be said that those plants are most nearly related which correspond in the greatest number of parts, and those most distantly in which we find the fewest points of correspondence". It is impractical to consider every detail of organization of every plant. "Hence the relative value of characters forms a most important part of the study of the Botanist". Yet, "all constant characters of whatever nature require to be taken into account in classifying plants according to their natural affinities". In his Introduction, which well repays careful reading as a whole and is written in a clear, somewhat robust, and rather attractive style, Lindley has many illuminating and suggestive dicta. A few more quotations are especially relevant to this paper. "Some writers, indeed, maintain that there cannot be more than one really natural system, any more than one planetary system; and in a certain sense this may be true, inasmuch as we must suppose that one plan only has been observed in the creation of living things, and that a natural system is the expression of that plan. But, on the other hand, it must not be forgotten that such a plan may be represented in many ways". "In natural science, the mode of arranging matter is susceptible of infinitely more variation than history; . . . . It is impossible, from the nature of things, that any arrangement should exist which shall represent the natural relations of plants in a consecutive series. It is generally admitted . . . that each species is allied to others in different degrees, and that such relationship is best expressed by rays (called affinities) proceeding from a common centre (the species). In like manner, in studying the mutual relationship of the several parts of the Vegetable Kingdom, the same form of distribution constantly forces itself upon the mind; Genera and Orders being found to be apparently the centre of spheres, whose surface is only determined by the points where the last traces of affinity disappear". "An arrangement, then, which shall be so absolutely

correct an expression of the plan of nature as to justify its being called *the* Natural System is a chimaera. All that the Naturalist can do is to carry into effect the principles above explained, with a greater or less amount of skill; the result of which will be *a* Natural System". All groups grade into one another and "are in one sense artificial, inasmuch as Nature recognises no such groups. Nevertheless, consisting in all cases of species very closely allied in nature, they are in another sense natural. But as the Classes, Sub-classes, Alliances, Natural Orders, and Genera of Botanists, have no real existence in nature, it follows that they have no fixed limits, and consequently it is impossible to define them. They are to be considered as nothing more than the expression of particular *tendencies* (nixus), on the part of the plant they comprehend, to assume a particular mode of development. Their characters are only a declaration of their prevailing tendencies".

The concept of an archetype by authors prior to 1859, seems, from the modern standpoint, sometimes to approximate closely to evolutionary ideas, and it is, indeed, sometimes difficult to realize that evolution was not then an accepted biological theory. T. H. Huxley (238), however, publishing in 1853, seems to oppose the theory of archetypes to what we should now call evolutionary views, when he says: "the archetypal cephalous mollusk . . . is . . . sharply separated from all other archetypes, whatever apparent resemblances or transitions may exist. In all cases these will, I believe, on close examination, be found to be mere cases of analogy, not of affinity. . . . If, however, all cephalous mollusks, *i.e.,* all cephalopods, gasteropods, and Lamellibranchiata, be only modifications by excess or defect of the parts of a definite archetype, then, I think it follows as a necessary consequence, that no anamorphism takes place in this group. There is no progression from a lower to a higher type, but merely a more or less complete evolution of one type.

"It may indeed be a matter of very grave consideration whether true anamorphosis ever occurs in the whole animal kingdom. If it do, then the doctrine that every natural group is organized after a definite archetype, a doctrine which seems to me as important for zoology as the theory of definite proportions for chemistry, must be given up." The word anamorphosis here apparently means a gradual change of form in a group in the course of geological time.

The two volumes published by Godron (164) on species and races are of particular interest as they represent the standpoint of many biologists immediately prior to the publication of Darwin's "Origin of Species". He clearly states the dogma of the constancy of species, and though races of domesticated animals and cultivated plants may vary greatly, they do not, according to Godron, produce new species. Even at the species level there can be no tracing of phylogeny.

Darwin

The contrast between the standpoint represented by Godron's two volumes and that expressed in Darwin's "Origin of Species" is remarkably great, the more so that both works were published in 1859 and were based on evidence much of which was essentially of similar type. The more one reads Darwin's works and ponders over them, and this applies above all to "The origin of species" and "Animals and plants under domestication", the more one realizes the tremendous depth of knowledge and original thought incorporated in them. The pre-Darwinian history of systematic botany, very briefly sketched above, shows clearly that evolutionary or phylogenetic ideas had no place in the construction of natural systems of classification. It may be difficult to understand exactly what many of the words and phrases regarding an order of nature, affinity, archetype, and so on, meant to biologists before 1859, the more so that many of these words and phrases were taken over by evolutionists with a change in explanation and then, more or less gradually, in connotation. It is, however, perfectly clear that the natural systems culminating in that of de Candolle were based on a comparative study of resemblances and differences, almost entirely of a gross morphological nature, and by grouping together plants, and smaller groups into larger groups, according as they showed the greater number of characters in common. The more of such characters two kinds or groups had in common, the closer was their affinity in the order of nature, which was the order not of their appearance in a geological time sequence but of an order established, presumably once and for all, when creation occurred. To read evolutionary or phylogenetic meanings into words and phrases abstracted and isolated from the published botanical works of pre-Darwinian systematists is, in general, misleading. Even the few exceptions (Adanson, Lamarck) appear to have had no influence upon the

actual making or, as the authors themselves would presumably prefer to say, discovery of what they considered the natural system.

We are not here concerned with the details of the very important Theory of Natural Selection. The speedy acceptance by the majority of biologists after 1859 of the general theory of evolution as a result of Darwin's work is the fact of primary importance, and its influence on general botanical theory can not be overestimated. Its significance in affecting taxonomic practice was not nearly so striking, partly because the establishment of natural systems was pre-Darwinian, partly because of difficulties in applying evolutionary concepts to classification—difficulties which are discussed later in this paper. It is, however, essential to remember the debt Darwin owed to the natural systems. It is difficult to believe that the theory of evolution, at least as a general theory, could have been established previously to the formulation of a natural system. That the natural system (or natural systems) received explanation through the acceptance of evolutionary theory and also through the theory of natural selection is obvious. Darwin himself emphasizes this again and again. For example, he says (125): "It is generally acknowledged that all organic beings have been formed on two great laws—Unity of Type, and the Conditions of Existence. By unity of type is meant that fundamental agreement in structure which we see in organic beings of the same class, and which is quite independent of their habits of life. On my theory, unity of type is explained by unity of descent. The expression of conditions of existence, so often insisted on by the illustrious Cuvier, is fully embraced by the principle of natural selection". Chapter XIII. of the "Origin of Species", on "Geographical Distribution", could not have been written in anything like its existing form except on a basis of a natural system, *i.e.,* a system formed on a consideration of the ensemble of characters. The first part of the penultimate chapter deals with classification and should be read in its entirely. Here, a few abstracts must suffice: "The grand fact of the natural subordination of organic beings in groups under groups, which, from its familiarity, does not always sufficiently strike us, is in my judgment thus explained [by divergent descent from a common parent]. No doubt organic beings, like all other objects, can be classed in many ways, either artificially by single characters or more naturally by a number of characters. We know, for instance, that minerals and

the elemental substances can be thus arranged.   In this case there is of course no relation to genealogical succession, and no cause can at present be assigned for their falling into groups.   But with organic beings the case is different, and the view above given accords with their natural arrangement in group under group; and no other explanation has ever been attempted".   He notes that: "the less any part of the organisation is concerned with special habits, the more important it becomes for classification. . . . certain morphological characters which are not functionally important . . . are often of the highest service in classification.   This depends on their constancy throughout many allied groups; and their constancy chiefly depends on any slight deviations not having been preserved and accumulated by natural selection, which acts only on serviceable characters".   He emphasizes the importance of correlation of characters.   One would have liked an enlarged discussion from Darwin on the sentence: "Geographical distribution has often been used, though perhaps not quite logically, in classification, more especially in very large groups of closely allied forms".   He believes the facts to show that "the Natural System is founded on descent with modification;—that the characters which naturalists consider as showing true affinity between any two or more species, are those which have been inherited from a common parent, all true classification being genealogical; that community of descent is the hidden bond which naturalists have been unconsciously seeking, and not some unknown plan of creation, or the enunciation of general propositions, and the mere putting together and separating objects more or less alike".   "I believe that the *arrangement* of the groups within each class in due subordination and relation to each other, must be strictly genealogical in order to be natural; but that the *amount* of difference in the several branches or groups, though allied in the same degree in blood to their common progenitor, may differ greatly, being due to the different degrees of modification which they have undergone, and this is expressed by the forms being ranked under different genera, families, sections, or orders".

Post-Darwinian Systems

The post-Darwinian history of systematic botany, the methods of obtaining classificatory and phylogenetic data, the validity or invalidity of phylogenetic theory and speculation, and the actual and

possible relationships between phylogeny and taxonomy occupy the remainder of this paper.   A few brief remarks on the principal systems proposed for plants, especially for the seed-plants, since 1859 will conclude this historical part.   Criticisms of systems purporting to be phylogenetic are partly embodied in the later parts.

The system of Bentham and Hooker (39) is, as already remarked, based very closely indeed on that published by de Candolle, so far as concerns the acceptance of families (128, 129) and their sequence. To this extent it is natural in the original meaning of the term as understood by de Candolle, but is not intentionally phylogenetic. L. Huxley (237) says: "Had the order of events been changed by ten years, and the planning of the 'Genera Plantarum' followed instead of preceding the 'Origin', it would have been arranged to show as far as possible a grouping by lines of descent.   But the original scheme had been worked out before the 'Origin' appeared, and it was not till nearly six years afterwards that Bentham confessed himself a complete convert".   Jackson (241), however, notes that in October 1858 we find Bentham "sketching out plan for Genera Plantarum with J. D. H[ooker]", and it was not till December 1861 that he received the proofs of the first sheet of the "Genera".   One suspects that it was rather the difficulties of agreeing on a phylogenetic system, and especially Bentham's doubts, and of expressing phylogenetic concepts in a linear classification that, probably wisely, prevented the publication of a phylogenetic system, rather than the time factor.   This is confirmed by a remark in one of Hooker's letters to Dr. E. N. Arber (237) in which he says: "I do not share Engler's views as expressed in his classification and writings.   The classification is neither better nor worse in the abstract than De Candolle's (so-called), and is far more troublesome to apply for practical purposes.   I hold to Robert Brown's view of the orders being reticulately not lineally related".   The great value of the "Genera Plantarum" is in the detailed yet concise descriptions of all the families and genera of the seed plants as then known.   The standard of these descriptions has never been excelled in any general taxonomic work.

An outline of the Engler and Prantl system, for the phanerogams, was published in 1897 (140), and "In dieser Übersicht sind die Familien der Siphonogamen mit Rücksicht auf ihre Verwandtschaft so angeordnet, wie es jetzt nach Abschluss ihrer Bearbeitung am

zweckmässigsten erscheint". Even in 1897, however, it is clear that Engler did not consider his system as phylogenetic, in the complete sense of the word, but rather as one in which the groups are built up in a step-like manner to form, as far as possible, a generally progressional morphological series. Some of the groups are acknowledged to be probably polyphyletic. No essential change in the general basis of the classification is recorded in later publications, but the discussions on principles make it increasingly clear that the system is intended to be only partially phylogenetic—that, indeed, a system composed of groups formed on resemblances and differences and then arranged hierarchically by combinations of progressions (see below), can not do more than include phylogenetic facts or suppositions in other than a patchlike manner. Apart from differences of opinion, some very great, as to the morphogenetic validity of the accepted progressions, there are innumerable difficulties in combining these, since they are often not parallel and frequently do not keep in step. It is, indeed, very true that "verschiedene Kombinationen von Progressionen erschweren die systematische Anordnung". Engler's conclusion (141) is: "So sehr ich mir auch von phylogenetischen Bestrebungen bei dem Studium einzelner Familien namentlich mit Zuhilfsnahme der Pflanzengeographie Erfolg verspreche, so stehe ich doch vielen Versuchen, Familien voneinander, von lebenden oder ausgestorbenen ableiten zu wollen, skeptisch gegenüber. Was man aber mit grösserer Sicherheit feststellen kann, das ist die Zugehörigkeit zu einer Familiengruppe oder Unterreihe und vor allem die morphologische Stufe. Mancherlei Verbesserungen bisheriger Anschauungen dürften sich aus weiterer Berücksichtigung der Haploidgenerationen und bei vorsichtiger Berücksichtigung serodiagnostischer Untersuchungen ergeben".

Bessey (43, 44) considered his system as "The Phylogenetic System of Flowering Plants". In saying "affinity is consanguinity and classification, so far as it is natural, expresses real relationship", he gives the most extreme phylogenetic interpretation to natural classification.

Hallier's various papers (189, 190, 191, 192, 193, 194, 195, and further references in these) suffer from being sometimes verbose, like some of their titles, and frequently written in a rather disconnected manner. He acknowledges, from time to time, changes of

opinion, and it is preferable to consult his later papers for a general view of his system (194, for an outline of the system; and 195, for the reasons for his classification and for references). Senn's excellent summary (387) of the basic principles of Hallier's system should also be read. Wangerin (477) also evaluates the characters used by Hallier. Hallier's system definitely purports to be phylogenetic.

Wettstein (484), unlike Bessey and Hallier, includes the whole plant kingdom, cryptogams as well as phanerogams, in his system. While his system is considered to be, as far as possible and bearing in mind the other aims of classification and the limitations imposed by a linear arrangement, in accord with phylogenetic principles, as he interprets them, he appears to realize more than many phylogenists the impossibility of a system which is both phylogenetic and generally useful. He says that "der phylogenetische Werth eines Systemes nur auf Kosten der Uebersichtlichkeit gewonnen werden kann". Wettstein's presentation of his system ranks high for clarity, conciseness and precision, and his book (several editions) is one of the best, if not the best, handbook of systematic botany— apart from any acceptance of his views on phylogeny and morphogeny.

Vuillemin (472) says that the application of the principle of affinity, based on characters, allows a juxtaposition in a chain (concatenation, series), which is logical, and the construction of a static system, which is rational. The principle of filiation gives a natural classification, symbolized by a genealogical tree. He points out difficulties of language (glossology) which are not always realized; thus, to describe plants as "apetalous" involves a comparison with their supposed higher derivatives; the term "incomplete" with reference to a flower postulates some mystic ideal type of flower. In his proposed classification he places the base of the dicotyledons at the level of the heterosporous Lycopodiales, and, in the absence of relevant palaeontological data, places first those plants uniting the greatest number of indices of inferiority—especially the Amentaceae from which he apparently derives most of the other existing dicotyledons.

Hutchinson's scheme (229, 230) is limited to the angiosperms, and these are, to quote the subtitle of his book, "arranged according to a new system based on their probable phylogeny". The

"general principles adopted for the classification" are "given" from Bessey "together with some additional observations", though the resulting system is different from Bessey's in some important respects.

Pulle (344) has published a classification of seed-plants which is based on Engler's system with certain modifications, the most important of which is the division of the Metachlamydeae (Sympetalae) into a number of groups which are attached to different series of Archichlamydeae.

In this general outline of the history of plant taxonomy, emphasis is intentionally given to those aspects which need relating to phylogenetic research and theory. In the course of the survey, though from details not enumerated above, it has become evident that the principal advances in taxonomy are due to three factors: *a*) improvement in taxonomic technique, *e.g.*, more adequate descriptions, better dissections, larger series of specimens examined; *b*) increase in systematic exploration (Linnaeus, Sp. Pl. 1753, described 5322 Phanerogams and 617 "Cryptogamia", the latter including a few animals; now some 250,000 species of seed-bearing plants have been described, and about 2,000 new, or supposedly new, species are being described every year, on an average); *c*) inclusion of results from modern advances in other biological subjects, *e.g.*, morphology, anatomy, ecology, phytogeography, genetics and cytology.

(Parts II and III of this study will appear in later numbers of the Review)

AMPHIDIPLOIDY

T. H. GOODSPEED AND MURIEL V. BRADLEY
Department of Botany, University of California

Investigation of the incidence and significance of amphidiploidy has recently been stimulated by the discovery of techniques which induce chromosome doubling and by improvements in their application. In fact, of the known instances of amphidiploidy at least one-third have been reported since 1937 and have been artificially produced.

Although the term "amphidiploid," when used literally, refers only to those organisms which possess in their somatic cells the diploid complement of both the parental species of an F_1 hybrid, this review will also refer to allopolyploids possessing more than two genoms of one or both of the species involved. Various synonyms for "amphidiploid" may be found in the literature—"tetraploid hybrid," "didiploid," "double diploid" and "allotetraploid" among others. Of the proposed systems of terminology for allopolyploid types, perhaps that of Warmke and Blakeslee (210) is[1] the most consistent and descriptive. According to this scheme, the F_1 hybrid is a "double haploid" and becomes a "double diploid" after chromosome doubling; with reduplication of the chromosome number, the term "double tetraploid" is applied, and so on. Similar logical terms are employed to designate polyploids in which different degrees of doubling in the two parental genoms have taken place.

At least as early as 1881 constant species hybrids were known. In that year Focke described *Aesculus carnea,* the true-breeding hybrid of *A. hippocastanum* and *A. pavia,* and Skovsted (186) has shown that the constancy of the hybrid is due to chromosome doubling. In 1891 Rimpau reported a constant hybrid in a cross between *Triticum* sp. and *Secale cereale,* the true-breeding properties of which were ascribed to amphidiploidy by Lindschau and Oehler in 1935. Janczewski in 1892 obtained a non-segregating

form of the hybrid *Anemone sylvestris* × *A. magellanica;* investigation may prove this to be an amphidiploid. *Rosa Wilsoni,* an allohexaploid of the cross *R. pimpinellifolia* × *R. tomentosa,* was described as such by Blackburn and Harrison in 1924; another allohexaploid, *Betula verrucosa* × *B. pubescens,* was reported by Helms and Jørgensen in 1925.

Winge's hypothesis (213) provided special significance to investigation of amphidiploidy and its rôle in species formation. The arithmetical progression of chromosome numbers in the various species of certain genera suggested to Winge that interspecific hybridization and subsequent chromosome doubling supplied an explanation of one mode of species origin. Clausen and Goodspeed (29) provided supporting evidence in their analysis of the amphidiploid *Nicotiana digluta* which originated from chromosome doubling in F_1 *N. Tabacum* × *N. glutinosa.* Additional evidence was furnished by the investigations of Newton and Pellew (147) on the origin of fertile individuals found in populations of *Primula kewensis,* the hybrid of *P. floribunda* and *P. verticillata.* Farmer and Digby in 1914 had reported 36 somatic chromosomes in the fertile *P. kewensis* in contrast to 18 in the sterile F_1, and Newton and Pellew provided the correct explanation of this chromosome duplication and applied Winge's hypothesis to explain the origin of *P. kewensis.*

ORIGIN OF AMPHIDIPLOIDS

Whether occurring in nature or artificially produced, amphidiploids arise as the result of hybridization followed by chromosome doubling or of fusion of diploid gametes from different autopolyploid forms. Chromosome doubling can take place at various points in the ontogenetic cycle. In F_1 meiotic divisions it may result from non-conjunction of chromosomes plus inadequate development of the spindle, causing failure of chromosome distribution to the two poles and subsequent formation of one rather than two nuclei. This single nucleus then contains the haploid complements of both parents. Following normal chromosome behavior in the second division, each of the two nuclei, and pollen grains, comes to possess the full somatic complement of the hybrid. Comparable phenomena in embryo sac development lead to the formation of female gametes also with the full somatic complement. Such chromosome doubling has been termed "non-reduction" by

Belling (13), "semi-heterotypic division" by Rosenberg (166), and in a later paper of Rosenberg's (167) "regression." Obviously, in order that an amphidiploid may be created directly from this process, both male and female gametes must be unreduced. Non-reduction in one type of gamete in each of two successive generations, when combined with backcrossing, will lead indirectly to amphidiploidy. Amphidiploids reported as having originated through fusion of unreduced gametes include *Aegilops ovata* × *Triticum turgidum* (155). *Triticum dicoccoides* × *Aegilops ovata* (95), *Triticum turgidum* × *T. villosum* (15), *Triticum vulgare* × *Secale cereale* (124), *Digitalis ambigua* × *D. purpurea* (25), *Crepis rubra* × *C. foetida* (159), *Erophila* sp. × *E. violacea-petiolata* (215), *Nicotiana rustica* × *N. paniculata* (117, 118, 180), *Nicotiana Tabacum* × *N. sylvestris* (171), *Phleum pratense* × *P. alpinum* (62), *Raphanus sativus* × *Brassica oleracea* (81), *Saxifraga adscendens* × *S. tridactylites* (39) and *Saxifraga rosacea* × *S. granulata* (212). Diploid gametes may also result from "non-division" (13), a term applied to failure of the second rather than the first meiotic division.

Other meiotic processes leading to unreduced gamete formation have been reported. Karpechenko (81) referred to the possibility that diploid sporocytes might be formed through failure of an archesporial nucleus to complete division after splitting of the chromosomes. Conjugation of homologues could then take place at meiotic prophase, leading to normal continuation of meiosis and the ultimate production of diploid gametes. Here again amphidiploidy would be dependent on the fertilization of an unreduced egg by an unreduced male gamete. Archesporial doubling was apparently the cause of chromosome duplication in the hybrid *Fragaria bracteata* × *F. Helleri* (75). The production of tetraploid gametes in the hybrid *Raphanus sativus* × *Brassica oleracea* was reported by Karpechenko (81). In the archesporial division immediately preceding meiosis, mitosis was not accompanied by cytokinesis. The two nuclei thus formed maintained their identities throughout meiotic prophase. The spindles fused at metaphase but there was no assortment of chromosomes to the poles, and a single nucleus with the tetraploid chromosome number was constituted. As a result of regular behavor of chromosomes at the second division, two tetraploid nuclei were formed. Kostoff (105)

assigned the probable origin of the amphidiploid *Nicotiana glauca ×
N. Langsdorffii* to parthenogenetic development of a "monad."
Equally possible, however, is its origin from parthenogenetic de-
velopment of a tetraploid megaspore formed according to the
process described by Karpechenko.

Duplication of the somatic chromosome complement in F_1 hy-
brids is a source of amphidiploidy. When doubling occurs in the
zygote, the resulting plant is entirely amphidiploid. Similarly, in
certain types of proembryos chromosome doubling in the particu-
lar cell from which root and shoot originate will initiate a com-
pletely amphidiploid plant. Chromosome doubling immediately
or soon after fertilization probably accounts for the amphidiploids
Nicotiana glutinosa × N. Tabacum (27, 29, 195), *Brassica napus ×
B. campestris* (52), *Nicotiana glutinosa × N. tomentosa* (45, 55),
Nicotiana Tabacum × N. glauca (197) and *Rosa pimpinellifolia ×
R. tomentosa* (19). If the complement is duplicated in a single
cell in a somewhat later phase of embryo development, a periclinal
chromosomal chimera will be constituted. But only if the chimera
is such that reproductive tissue is descended from polyploid cell
lines, can amphidiploidy be perpetuated in the race. Through
somatic doubling in the meristem of a lateral bud of an F_1 hybrid,
ı amphidiploid branch can originate; Newton and Pellew (147)
·ported a case of this type in the F_1 *Primula floribunda × P. verti-
'ata,* and Ternovsky (198) cited a partially amphidiploid chro-
ˉmosomal chimera in F_1 *Nicotiana Tabacum × N. sylvestris.* So-
matic doubling in a vegetative bud is the source of many artificially
induced amphidiploids, resulting from application of colchicine or
other reagents to bud meristems. Somatic doubling may also ac-
company regeneration after wounding or decapitation, when groups
of adventitious meristematic cells are stimulated to polyploidy and
give rise to amphidiploid branches (77).

A more remote mode of amphidiploid origin lies in the hybridiza-
tion of two tetraploid plants. Because of multivalent formation in
autopolyploid meiosis, polysomic and deficient gametes are preva-
lent and lowered gametic viability follows. Also, the products of
tetraploid hybridization may be chromosomally aberrant, so that
perfect amphidiploids are not formed.

To summarize, the modes of amphidiploid origin are: *a*) non-
reduction in both male and female gamete formation, *b*) non-re-

duction in one type of gamete in each of two successive generations, combined with backcrossing, c) chromosome doubling in arche-sporial tissue, d) somatic doubling in the F_1 zygote or in the pro-embryo, e) somatic doubling in an F_1 vegetative bud or in connection with regeneration, and f) hybridization of two autotetraploid species.

The fertility of a newly discovered amphidiploid as compared with that of its hybrid parent may in some cases point to the type of amphidiploid origin (33). This applies especially to hybrids of distantly related species, where little or no pairing occurs between chromosomes of the two parental genoms, and the F_1 hybrid is competely or almost entirely sterile. In such case the formation of an occasional seed which gives rise to an amphidiploid plant suggests that non-reduction in both male and female gametogenesis or doubling in archesporial tissue has taken place. The presence of a fertile individual, all branches of which prove to be amphidiploid, in an otherwise sterile F_1 population obviously suggests doubling in the zygote or in an early embryonic phase. But if a fertile amphidiploid branch appears on an otherwise infertile hybrid plant, somatic doubling must have taken place in a vegetative bud.

EXPERIMENTAL INDUCTION OF AMPHIDIPLOIDY

Amphidiploidy has been experimentally achieved through certain types of hybridization. In F_2 of the hybrid *Galeopsis pubescens* × *G. speciosa* (139, 140, 141) a single triploid plant appeared as a consequence of non-reduction in the formation of one gamete. In backcrossing the triploid to *G. pubescens,* an unreduced egg of the triploid was fertilized by a reduced male gamete of *G. pubescens,* and a single seed was obtained which gave rise to the amphidiploid form, known as "artificial *G. Tetrahit.*" Taking advantage of non-reduction in gametogenesis of F_1 *Nicotiana rustica* × *N. paniculata,* Kostoff (97, 98) obtained several sesquidiploids—hybrid derivatives possessing one genom from one parent and two genoms from the other parent (211)—in the backcross of the hybrid to *N. rustica.* Backcrossing (*N. rustica* × *N. paniculata*) × *N. rustica* to *N. paniculata,* and through the agency of non-reduction in sesquidiploid gametes, he produced two amphidiploids. By the same technique Kostoff (97) obtained the amphidiploids *Nicotiana glauca* × *N. Langsdorffii, N. sylvestris* × *N. tomentosiformis (Rus-*

byi) and *N. Tabacum* × *N. tomentosa,* and Rybin (170) developed the amphidiploid *Nicotiana Tabacum* × *N. rustica.* Obviously, the greater the ratio of viable unreduced gametes to viable reduced gametes, the more successful this method of constructing amphidiploids is likely to be. The few seeds obtained from plants in which only unreduced gametes are viable will give rise to a high percentage of polyploids, whereas if reduced gametes are viable, more seed may set and produce a variable population yielding few polyploids. For this reason the synthesis of the sesquidiploid plant is accomplished with greater facility than that of the amphidiploid; the sesquidiploid plants produce a large percentage of reduced, and frequently aberrant, viable gametes, which necessitates the growing of large populations in the succeeding generation and the cytological examination of numerous plants in order to segregate the amphidiploids.

A somewhat different type of amphidiploid induction is represented by the amphidiploid *Triticum Timopheevi* × *T. monococcum* which Kostoff (101) obtained by crossing the F_1 to *T. duroturgidoide.* Actually, this third species took no part in amphidiploid formation aside from influencing apomictic development of the hybrid by the stimulating action of the pollen tube. Kostoff (105) interpreted the origin of amphidiploid *Nicotiana glauca* × *N. Langsdorffii* as a result of parthenogenesis activated by the application of *N. Langsdorffii* pollen to the stigma of the F_1 hybrid. An amphidiploid *Triticum dicoccum* × *Haynaldia villosa* was similarly obtained through the stimulating action of pollen of a third species, *T. Timopheevi,* on the stigma of the F_1 hybrid (114).

Segregation products from the selfing of sesquidiploid hybrids may include some amphidiploids; a case in point is the amphidiploid *Crepis capillaris* × *C. tectorum* obtained by Hollingshead (70) in this way. A sesquidiploid appearing in the F_1 population possessed two *capillaris* genoms, due to non-reduction. Progeny of the selfed sesquidiploid included a variety of segregates among which was the amphidiploid. The probability of obtaining a perfect amphidiploid by this method is limited, except in species which have low chromosome numbers, as in these *Crepis* species, *C. capillaris* with 3 pairs, *C. tectorum* with 4 pairs.

Hybridization of two autotetraploids may establish new amphidiploids, that of two autohexaploids new amphitriploids, *etc.* An-

derson (4) produced the amphidiploid *Tradescantia subaspera* × *T. canaliculata* by crossing tetraploid forms of these species, and an allotetraploid, *Lycopersicum pimpinellifolium* × *L. esculentum*, was obtained by hybridizing these tetraploid *Lycopersicum* species (131). Theoretically, through hybridization of a pentaploid or hexaploid with a tetraploid, amphidiploids may be obtained in segregation products of inbreeding, due to loss of chromosomes of the extra genom or genom fraction. Likewise, crosses between species with higher multiples of the basic genom—heptaploid, octoploid, *etc.*—might give rise through segregation to new polyploid forms with the same number of genoms from each parental species. Actually, because of structural chromosomal changes which have occurred in polyploid species, especially in those of remote origin, the original genoms may have become so altered that hybridization of these polyploids will not give rise to amphidiploids, -triploids, *etc.*, in the strict sense of the word. Many of the polyploid hybridization products cited in the literature are of this order, particularly the *Triticum* and *Aegilops* allopolyploids.

Chromosome doubling frequently accompanies regeneration of plant organs. Winkler in 1916 employed this method of producing tetraploid shoots in *Solanum*. Jørgensen (77) developed the amphidiploid *Solanum nigrum* × *S. luteum* by decapitation of an F_1 plant, some of the shoots arising from the callus formed on the cut surface having the doubled chromosome number. The amphidiploid form of *Pelargonium radula roseum*, a hybrid of unknown parentage, was also obtained through regenerative processes (179). Karpechenko (84, 85) obtained amphidiploids by decapitation and regeneration in the hybrid *Raphanus sativus* × *Brassica oleracea*, and an amphidiploid *Lycopersicum pimpinellifolium* × *L. esculentum* was produced from callus shoots by Lindstrom (129). Application of indole-3-acetic acid in lanolin paste to cut surfaces of decapitated plants has been found by Greenleaf (58, 59, 60) to be a rather effective means of inducing chromosome doubling; by this method amphidiploids of F_1 *Nicotiana glutinosa* × *N. tomentosa*, *N. sylvestris* × *N. Setchellii*, *N. sylvestris* × *N. tomentosa* and *N. sylvestris* × *N. tomentosiformis* were obtained. This technique is of particular value when attempts are made to induce chromosome doubling in species that do not readily form abundant callus. Other substances (84) have been used in connection with regenera-

tion to cause duplication of the chromosome complement, among them plant extracts containing wound hormones, extracts of orchid pollinia, mixtures of zinc or magnesium salts, and cultures of *Bacterium tumefaciens;* but it is doubtful that any of these has proved as effective as indole-3-acetic acid.

Various reagents have been used to induce polyploidy through processes other than those associated with regeneration, and of all these colchicine has proved to be the most important. Since the original investigations by Dustin, Havas and Lits (40) on colchicine effects, numerous reports of similar studies have appeared in the literature (*cf.* Dermen [35]). Among the amphidiploids obtained by the various colchicine techniques are *Aegilops caudata* × *A. umbellulata, Aegilops speltoides* × *A. umbellulata* and *Triticum monococcum* × *Aegilops uniaristata* (178); *Cucurbita maxima* × *C. moschata* (21); *Gossypium arboreum* × *G. thurberi* (12); *Gossypium hirsutum* × *G. Sturtii* (219); *Hyoscyamus niger* × *H. albus* (64); *Mentha aquatica* × *M. rotundifolia* (146); *Nicotiana glutinosa* × *N. sylvestris* (21); *Nicotiana glutinosa* × *N. Tabacum* (135); *Nicotiana Setchellii* × *N. otophora* (22); *Nicotiana suaveolens* × *N. alata* and *Nicotiana excelsior* × *N. velutina* (106); *Nicotiana Tabacum* × *N. glauca* (187, 188); *Nicotiana Tabacum* × *N. sylvestris* (187, 10); and *Solanum melongenum* × *S. tamago* (150).

The primary effect of colchicine on the cell is inhibition of spindle formation so that the chromosomes remain in metaphase condition for a longer period of time than normally (11, 17). Furthermore, complete division of the chromosomes is inhibited by the failure of the centromere to divide when the rest of the chromosome divides, another factor which prevents anaphasic movement of the chromosomes. After sufficient recovery from the effects of colchicine, division is completed and the divided chromosomes reorganize into a single nucleus with the doubled chromosome number. Or, if colchicine penetrates the cell at anaphase in concentration sufficient to inhibit normal completion of mitosis or meiosis, the two groups of anaphase chromosomes may fuse to form a single nucleus. Succeeding cell generations then contain the doubled chromosome complement. The apparent cause of failure of spindle formation is decrease in cytoplasmic viscosity; thus the normal viscosity changes which accompany mitosis and meiosis are inhibited (11). Bhaduri (17) believes that colchicine acts as a cata-

lyst, setting up abnormal reactions in the cytoplasm, and that these reactions presumably bring about physical changes in the protoplasm. Like most catalytic reactions, that of colchicine is reversible, which accounts for the ultimate recovery of treated cells.

Certain aberrations in meiosis or mitosis may occur in cells that have partially recovered from colchicine. The abnormal groupings of chromosomes in metaphase may cause more than one spindle to form, resulting in the production of several daughter cells with various chromosome numbers (11, 125). Likewise, aneuploidy may be a consequence of partial instead of total mitotic inhibition. Cases of chromosome breakage caused by colchicine were reported by Eigsti (43, 44). Because of these induced abnormalities, colchicine treatment of hybrids frequently gives rise to forms not completely amphidiploid.

Eghis (42), using chloroform, obtained an amphidiploid *Nicotiana Tabacum* × *N. sylvestris;* among many treated flowers, only one responded to chloroform treatment by chromosome doubling. Kostoff (110) demonstrated that chromosomes may be doubled by treating plants with acenaphthene. Levan (126) has studied the effects of acenaphthene on mitosis and found that its reaction is slow, lasting for several days. Acenaphthene brings about the same deviation from normal mitosis as colchicine; but whereas colchicine, except in very dilute solutions, inactivates the exterior and interior parts of the spindle simultaneously, acenaphthene causes a differential action, the exterior portion reacting first. Because of the slow action of acenaphthene, a predisposition to formation of aneuploid cells occurs. Among other reagents which in proper concentration interfere with mitosis without lethality are chloral hydrate, certain arsenic and lithium compounds, some dyestuffs, naphthalene compounds, and phenanthrenes (146). It is possible that some of these might be equally as effective as colchicine in producing amphidiploids from F_1 hybrids.

The effects of temperature extremes may induce polyploidy. Probably the first polyploids produced by high temperature treatment were those of Randolph (162) who obtained some tetraploid corn plants as the result of heat application to zygotes in the single-celled stage. Sax (177) has pointed out that, whereas in certain genera moderate temperature changes are sufficient to cause chromosome doubling, more effective results may be obtained by sub-

jecting plants to a preliminary cold treatment before application of heat. Karpechenko (85) noted that in F_1 *Brassica oleracea* × *B. carinata* chromosome doubling frequently coincides with a considerable lowering of temperature, and Kostoff and Radjably (116) believed that cold increased the percentage of unreduced gametes in F_1 *Nicotiana rustica* × *N. paniculata*. One disadvantage of temperature-treated material is that fragmentation, fusion and bridging are likely to occur, probably as the result of chromosome clumping induced by temperature extremes (34). Among the amphidiploids obtained through temperature shock are *Nicotiana multivalvis* (*N. Bigelovii*) × *N. suaveolens* (102), *Triticum vulgare* × *Secale cereale* (38), *Triticum vulgare* × *Agropyron glaucum* (156) and *Nicotiana Tabacum* × *N. sylvestris* (198).

Beams and King (11) have shown that centrifuging may cause metaphase chromosomes to be thrown free of the spindle, thus inhibiting normal continuation of mitosis or meiosis; this action is comparable to that of colchicine and likewise prevents anaphasic movement of the chromosomes. By centrifuging germinating seeds of hybrids between various strains of *Nicotiana Tabacum* and *N. sylvestris*, Bartolucci (10) obtained a few amphidiploids. Of plants from treated seeds, .08% showed the effects of chromosome doubling. This method is relatively inefficient, as shown by the fact that colchicine-treated seeds of the same hybrids produced .29% amphidiploids. In a population grown from 81 centrifuged seeds of *Nicotiana rustica* × *N. Tabacum*, Kostoff (103) found a single plant in which the chromosome number had been altered by treatment; this plant was a chromosomal chimera with one amphidiploid branch. Although no amphidiploids have been reported as originating from effects of high frequency radiation, Goodspeed (53) obtained tetraploid *Nicotiana Tabacum* as the result of x-ray treatment of a diploid plant. Therefore, this technique can not be overlooked as a possible means of inducing amphidiploidy.

THE ORIGIN AND CHARACTER OF NATURALLY OCCURRING AMPHIDIPLOIDS

Most known amphidiploids have been artificially created or have appeared spontaneously in hybrid cultures. However, for several species occurring in nature amphidiploid origin has been postulated and has been substantiated by fairly conclusive evidence. With

foundations already laid for the investigation of origin of species with multiples of a basic chromosome number, many additional species will undoubtedly prove to be allopolyploids.

From knowledge of agencies successfully used in experimental induction of chromosome doubling, some of the conditions responsible for the origin of natural amphidiploids may be postulated. Artificial production of polyploids by means of temperature shock suggests a similar effect of heat and cold in the case of plants growing in the wild. Origin of amphidiploid shoots from hybrid callus tissue after deliberate decapitation probably has a parallel in regenerative processes following injury to natural hybrids. Nutritional deficiencies may account for some instances of allo- and autopolyploidy. According to Whyte (212), if the necessary nutritional supply is not available to all cells in the anther, some nuclei undergoing the first meiotic division may show impairment in the separation and passage of the chromosomes to the poles, and non-reduction results. Whyte attributed chromosome doubling in F_1 *Saxifraga rosacea* × *S. granulata* to nutritional deficits.

The hybrid state *per se* is perhaps the most important single factor in the origin of natural amphidiploids. This is indicated by the high percentage of restitution nuclei observed in hybrid gametogenesis in comparison with that found in species gamete formation. Where incompatibility between the two genoms in a hybrid is so great that little or no chromosome pairing occurs, there is a tendency toward chromosome doubling (213).

In addition to the natural amphidiploids mentioned in the introduction, there is *Galeopsis Tetrahit,* the origin of which is to be assigned to chromosome doubling in the hybrid *G. pubescens* × *G. speciosa,* two species with the haploid chromosome number of 8 (139, 140, 141). Experimental evidence for the origin of *G. Tetrahit* was obtained by synthesizing it from the two 8-paired species. The striking resemblance of this artificial form to the natural *Tetrahit* is indicative of the genesis of the latter from a cross between *G. pubescens* and *G. speciosa* or their progenitors. The artificial form resembles the natural not only in external morphology, but also in cytological behavior, each having 16 pairs of chromosomes which behave regularly in meiosis. Moreover, when the natural and artificial types are crossed, the resulting progeny is composed of normal individuals similar to both parents.

Another species of presumable allopolyploid origin is *Spartina Townsendii*. Huskins (72) presents evidence that *S. stricta*, a native European species, and *S. alterniflora*, an American species introduced into England, were the parents of a hybrid which, after chromosome doubling, became *S. Townsendii*. Chromosome numbers in the diploid species and their amphidiploid derivative corroborate morphological evidence—the gametic chromosome number of *S. stricta* is 28, that of *S. alterniflora* 35, whereas *S. Townsendii* possesses the sum of the chromosome numbers of these postulated ancestral forms. In view of the high chromosome numbers of *S. stricta* and *S. alterniflora*, these species themselves may have a background of allopolyploidy, in which case *S. Townsendii* could not be considered a simple amphidiploid.

Considerable evidence for the amphidiploid origin of *Nicotiana Tabacum* has accumulated. Goodspeed and Clausen established the fact that a progenitor of the modern species *N. sylvestris* was undoubtedly involved in the hybrid from which the amphidiploid *N. Tabacum* originated, also that a progenitor of some member of the "tomentosa group" of the genus *Nicotiana* had served as the other parent (56a, 28, 55). The *Tabacum* genom is composed of two subgenoms of twelve chromosomes each, one of which is homologous with the *sylvestris* gametic set, the other with that of members of the "tomentosa group." Although chromosome pairing relations in hybrids of *N. Tabacum* and *N. tomentosa*, and of *N. Tabacum* and *N. tomentosiformis*, a species closely allied to *tomentosa*, are practically identical, with 12 pairs and 12 univalents the common meiotic metaphase condition in both hybrids, morphological evidence favored *tomentosiformis* rather than *tomentosa* as one of the species the progenitor of which entered into the amphidiploid origin of *N. Tabacum*. Recently, a series of new and little known members of the "tomentosa group" have been obtained in Peru, Bolivia and Argentina and grown in the University of California Botanical Garden (56). Preliminary evidence indicates that the progenitor of one of the recent accessions, *N. otophora*, rather than *N. tomentosa* or *N. tomentosiformis*, may represent the parent which, with *N. sylvestris*, was responsible for the amphidiploid origin of *N. Tabacum*. Certainly, F_1 *N. sylvestris* × *N. otophora* is morphologically more similar to *N. Tabacum* than the F_1 of *N. sylvestris* and other members of the "tomentosa group." In this

connection it is also to be noted that *N. otophora* has been found in the same geographical area as that occupied by *N. sylvestris,* something not true of other members of the "tomentosa group."

Nicotiana rustica is another amphidiploid species, the probable origin of which has been established as a hybrid between progenitors of the modern species *N. paniculata* and *N. undulata* (55, 111). This postulate is based in part upon *Drosera*-type chromosome pairing in F_1 hybrids of *N. paniculata* and *N. undulata* with *N. rustica.* Furthermore, trigenomatic *paniculata-undulata-rustica* plants were obtained, which closely resembled the natural *N. rustica;* these plants showed 24 chromosome pairs at meiosis and were fertile (55). Similarities in the chromosome morphology of *N. paniculata* and *N. rustica* and the limited genetic differences between these two species are cited by Kostoff (99) as indicating that *paniculata* progenitors participated in the origin of *N. rustica.* *Nicotiana Bigelovii, N. nudicaulis* and *N. repanda* are other species believed to have arisen through amphidiploidy (55). Little evidence is available respecting their origins, but it is probable that a progenitor of *N. attenuata* was involved in the production of *N. Bigelovii;* and it has been suggested that an ancestral form of *N. trigonophylla* was one of the species which entered into the origin of *N. nudicaulis.* A new species of *Nicotiana* recently found near the Bolivian border in southeastern Peru, possesses 24 pairs of chromosomes and on morphological grounds appears to be a naturally occurring amphidiploid, the origin of which can be referred to the progenitors of the modern species *N. undulata* which is largely Peruvian in distribution, and *N. wigandioides* which has been found only in Bolivia.

Among other natural allopolyploids for which similar evidence of origin is available are: *Iris versicolor,* believed to be an amphidiploid of *I. virginica* and *I. setosa* (3) ; the hexaploid species *Phleum pratense,* the derivation of which may have involved chromosome doubling in a hybrid of *P. nodosum* (the species name used by Müntzing [143], synonymous with diploid *P. pratense* [Group II] of Gregor and Sansome [62]) and a tetraploid species, possibly the tetraploid *P. alpinum* which may itself have originated through amphidiploidy ; *Pentstemon neotericus,* which J. Clausen (26) demonstrated to be the amphidiploid of *P. laetus* × *P. azureus;* and perhaps *Verbascum phoeniceum* (121), cytological evidence

for which points to amphidiploidy, although its probable parents have not been postulated.

Data on polyploid hybrids show that certain structural features commonly undergo modifications proportionate to the degree of chromosome doubling. Other characters, however, although influenced by duplication of the chromosome complement, fail to express consistent correlations with chromosome number changes. Size and vigor fall into the latter category. Amphidiploids are frequently larger and more robust than the F_1 hybrids from which they originate, as illustrated by those of F_1 *Nicotiana glutinosa* × *N. Tabacum* (29), *Nicotiana multivalvis* × *N. suaveolens* (102), *Mentha aquatica* × *M. rotundifolia* (169) and *Aquilegia chrysantha* × *A. flabellata* (184). Presumably, increased size is due to enlargement of all plant organs (179), which is in turn the product of increase in cell volume. By contrast, several instances of unmodified or even decreased size or vigor in amphidiploids may be cited. No appreciable variation from the corresponding undoubled F_1 hybrids was shown by the *Triticum-Aegilops* and interspecific *Aegilops* amphidiploids of Sears (178), by the allotetraploid *Nicotiana rustica* × *N. Tabacum* (103) or by the amphidiploid *Ocimum canum* × *O. gratissimum* (119). Actual dwarfing was reported by Kostoff (100, 100a) in the amphidiploid *Nicotiana rustica* × *N. glauca*. Dwarfness was also characteristic of some individuals in an amphidiploid *Nicotiana Tabacum* × *N. otophora* population (22). Kostoff assigned the lowered vitality of his dwarf amphidiploid to a too great increase in chromosome number. However, there may be other factors than high chromosome numbers which limit size development in amphidiploids. Segregation following multivalent association of chromosomes in meiosis may give rise to plants which, although they possess the exact amphidiploid chromosome number, have fewer than two chromosomes of one type and more than two of another. Certain of the dwarf *N. Tabacum* × *N. otophora* amphidiploids are probably of this nature. Qualitative chromosome alterations may likewise account for some instances of decrease in size and vigor. Also, genetic factors may suppress the robustness frequently associated with chromosome doubling.

Although amphidiploid and F_1 hybrid size relationships are inconstant, increased coarseness of the amphidiploid habit is to be expected (109).

Alterations in leaf structure are correlated with the doubling of chromosome number in hybrids. The combined investigations of a number of authors (74, 107, 103, 105, 184, 173, 187, 147) have shown that when compared with F_1 hybrids, amphidiploid leaves commonly show greater laminal thickness, increased breadth in relation to length, and coarser texture. They are usually darker green in color than the leaves of the corresponding diploid hybrids (12, 106, 109, 187), due in part to the greater thickness of leaf tissue in the polyploid forms. Increases in number of chloroplasts in the cell and in concentration of chlorophyll per unit area proportionate to the chromosome number increase in $2n$, $4n$ and $8n$ forms of the hybrid *Nicotiana alata* × *N. Sanderae* were reported by Kostoff (109). Kostoff and Orlov (115) found that if there is any size difference between chloroplasts of diploid and polyploid forms, those of polyploids are smaller; the same phenomenon was also reported by Grebinskaya (57).

The size of stomata and guard cells has been used as a criterion of chromosome doubling, since polyploid forms usually have larger stomata than diploids. Although this test has served in many cases (106, 107, 187, 12, 210), it is not infallible (35). In an amphidiploid *Triticum monococcum* × *Aegilops uniaristata* the stomata were no larger than those of the diploid (178). Warmke and Blakeslee (210), correlating stomatal size with fertility of colchicine-treated hybrids, found larger stomata in some sterile hybrids than in some fertile, presumably amphidiploid plants. According to their explanation, these discrepancies are due to the origin of germinal and epidermal tissues from different embryonic layers; thus chromosome doubling in epidermal tissue may occur without accompanying polyploidy in the underlying tissues, and the reverse situation may likewise exist. In keeping with general increase in cell size of many polyploids, trichomes have been found to be larger in certain amphidiploids than those of their parental F_1 hybrids (12, 106).

Although flowers of amphidiploids are frequently broader, and in some cases longer, than those of the corresponding undoubled hybrids (27, 172, 187, 210), this is not always the case. Single-

ton (180) and Lammerts (118) found little difference in flower size in F_1 hybrids and amphidiploids of *Nicotiana rustica* × *N. paniculata*. Other alterations in flower structure may accompany chromosome doubling. Smith (187) and Warmke and Blakeslee (210) noted changes in the relative lengths of style and stamens. Greater breadth of stigmas and styles frequently occurs as a result of chromosome doubling (109). Increase in volume of pollen mother cells and of pollen accompanies that of the nucleus when the chromosome number has been doubled. In general, pollen of amphidiploids may be expected to be larger than that of the corresponding diploid hybrids (106, 171, 172, 215, 210), and thus determination of pollen size is useful in detection of the amphidiploid condition. As a criterion of chromosome doubling this method is much more certain than comparison of stomatal sizes. Pollen tubes are thicker than those of corresponding F_1 hybrids (107) and are likely to develop more slowly, as noted by Ternovsky (198) in the amphidiploid *Nicotiana Tabacum* × *N. sylvestris,* which may partially account for the incomplete fertility of this amphidiploid. Kostoff (107) found that caryopses of the amphidiploids *Triticum vulgare* × *Secale cereale* and *Triticum durum* × *Secale montanum* were larger than those of the parental forms. Seed fibers of an octoploid *Gossypium* hybrid, which Beasley (12) obtained by colchicine treatment of a hybrid of two allotetraploids, were longer than those of either of the parents.

A decrease in rate of cell division has been noted in certain amphidiploids. This is especially noticeable in some colchicine-treated plants where portions of a leaf are composed of diploid cells, while in other portions the chromosome number has been doubled. The diploid areas rapidly outgrow the tetraploid, resulting in wrinkling and distortion of the lamina and in differences in leaf thickness at various points. The decreased growth rate may be responsible for the longer vegetative period which Kostoff (106, 107) noted in the amphidiploids *Nicotiana excelsior* × *N. velutina* and *Nicotiana suaveolens* × *N. alata.* Increased resistance to cold in the amphidiploid *Nicotiana rustica* × *N. glauca* as compared to the diploid hybrid was reported by Kostoff (113). On the other hand, evidence reviewed by Stebbins (192) indicates that tetraploid tissues show a higher water content than diploid tissues. This is associated with a lowered osmotic value in $4n$ cells and

causes the tetraploid to be less resistant to frost than the diploid.
Immunity from rust was found to be characteristic of the amphi-
diploid *Aegilops longissima* × *Triticum durum* (190).

CYTOGENETICS OF AMPHIDIPLOIDS

Two types of chromosome conjugation are found in amphi-
diploid meioses—autosyndesis and allosyndesis. Because of con-
fusion in the literature regarding these terms (*cf.* 33, 37, 96, 121,
132, 174), it is necessary to indicate our use of them by the fol-
lowing definitions. Autosyndesis refers to conjugation of chro-
mosomes descended from the same species or subspecies. Allo-
syndesis is conjugation of chromosomes descended from different
species or subspecies. Either type of conjugation implies some
degree of structural similarity—similar arrangement of genes—
between the chromosomes involved. Structural likeness may not,
however, invariably result in pairing, for failure of conjugation
may be genically conditioned (37). Autosyndesis, as defined
above, applies to pairing of chromosomes (*a*) of normal diploid
species, (*b*) of autotetraploid species, (*c*) derived from the same
gametic set in hybrids involving as either or both of the parental
forms species of remote autotetraploid origin, and (*d*) from the
same species or subspecies in an amphidiploid.

Allosyndesis is encountered in some F_1 hybrids and in some
amphidiploids. Theoretically, because of "differential affinity"
(33)—stronger attraction ordinarily manifested by a chromosome
for its identical partner than for other chromosomes to which it
shows only partial structural similarity—competition in conjuga-
tion leads first to autosyndesis in the amphidiploid, with allosyn-
desis playing a subordinate role and affecting pairing only after
the affinity between identical mates has been at least partially satis-
fied. Thus, in cases where differential affinity obtains, allosyn-
desis results in multivalent configurations.

Obviously, the distinction between auto- and allosyndesis is not
always clean-cut, and pairing in amphidiploids can not in all cases
be resolved into one or other of these two categories of chromo-
some conjugation. When the chromosomes of two parental spe-
cies are divergent structurally and no multivalents are formed, pair-
ing may usually be attributed to autosyndesis alone, while, as the
chromosomes of the parental species approach structural equiva-

lence, the distinction between allo- and autosyndesis breaks down. Another complicating factor is the possibility of random conjugation—allosyndesis partially replacing autosyndesis—giving rise to bivalents instead of the multivalent configurations commonly associated with allosyndesis (cf. 218). This might occur when certain chromosomes of the two parental species or subspecies are so completely homologous that no differential affinity exists among them and conjugation is at random, a phenomenon to be determined only by genetic studies, where genes carried by the homologues of the parental forms are allelomorphic. Moreover, the possibility of conjugation of unlike chromosomes must not be overlooked (cf. 131). Jørgensen (77) has also contended that the capacity for conjugation can not be considered a reliable measure of the degree of identity of the chromosomes entering meiosis, and that factors other than the chromosomes themselves may influence the process of conjugation. An additional complication might arise from the pairing of chromosomes within the same gametic set (33). This could not be classified as allosyndesis and could be considered autosyndesis only when one of the conjugating chromosomes represents a reduplicated chromosome, or in cases where one or both of the original parental species is of autotetraploid origin.

Conjugation in amphidiploids, particularly allosyndesis, may be influenced by length of chromosomes (90). In an amphidiploid of two closely related species, each of which possesses long chromosomes, the numerous chiasmata tend to hold together the chromosomes of a multivalent configuration. On the other hand, regardless of considerable homology between chromosomes of two species which form an amphidiploid, if the chiasma frequency of the parents is low, multivalents may be dissolved prior to metaphase, or the possibility of their formation in prophase be reduced. Amphidiploids with long chromosomes, derived from F_1 hybrids with a high rate of allosyndesis, have relatively little chance for survival (112), due to unequal distribution of chromosomes in anaphase.

The occurrence of allo- *versus* autosyndesis can at times be determined either cytologically or genetically. The presence of heteromorphic pairs may be indicative of allosyndesis. That this is not, however, a perfect criterion was pointed out by Kostoff (105), since segmental interchange occurring in the F_1 hybrid or

in previous generations of the amphidiploid might alter one or both of a pair of homologues to the extent that one chromosome of a bivalent is visibly larger than the other. Differences in staining properties of *Nicotiana glauca* and *N. Langsdorffii* chromosomes, due to differences in the extent of heterochromatic regions, were also used by Kostoff (105) in determining the occurrence of allosyndesis in the amphidiploid of these species. Data from this source indicated that some bivalents were probably the results of chromosome associations between heterochromatic regions of nonhomologous chromosomes. Genetic analysis of F_2 amphidiploid progenies or of populations derived from backcrossing amphidiploids to recessive $4n$ types gives evidence as to the category of syndesis involved (130). Collins and Longley (30) determined the percentage of auto- and allosyndesis in connection with a set of four homologous chromosomes in a tetraploid hybrid of maize and perennial teosinte and devised a formula for calculating the coefficient of autosyndesis.

In amphidiploids a certain structural similarity, not sufficient to result in true chromosome conjugation, is said to be indicated by what has been called "secondary pairing" (33, 90). No pairing of the chromosomes concerned occurs in prophase, but bivalents at MI or chromosomes at MII appear to become aligned with other bivalents or chromosomes, with the result that a condition resembling pairs or small groups of bivalents in MI or of chromosomes in MII may be seen. The significance of secondary pairing is merely that the origin of a species in which secondary pairing occurs may be attributed to polyploidy, if the chromosome number of the species is large enough to be considered a multiple of a basic genom. Other criteria must, however, be applied to determine whether the species in question is of allo- or autopolyploid origin.

As previously indicated, the type of conjugation in an amphidiploid is normally a function of the degree of structural equivalence of the two genoms involved. Thus in amphidiploids of distantly related species and in intergeneric amphidiploids autosyndesis should be the rule, following practically complete lack of pairing and the resulting sterility in the F_1 from which the amphidiploid is derived. Hence, only bivalents occur and distribution of the chromosomes to the two poles is comparable to that of a normal diploid species. Since all gametes thus formed possess the

full quotas of types and numbers of chromosomes, fertility is high and the amphidiploid progeny is uniform. Autosyndesis is well illustrated by pairing relations in the intergeneric amphidiploid *Raphanus sativus* × *Brassica oleracea* of Karpechenko (82, 83). Of the 18 bivalents in meiosis, 9 are formed through conjugation of the 9 chromosomes of the *Raphanus* gametic set with their 9 homologues, and the remaining 9 pairs are due to conjugation of the 9 *Brassica* chromosomes with their *Brassica* homologues. Cytogenetic studies of a *Raphanobrassica* amphidiploid were also made by Howard (71), whose results differed from those of Karpechenko because of some diversity in the strains of *Raphanus* or *Brassica*. The occurrence of a few trivalents, one quadrivalent and anaphase bridges led to the conclusion that one of the parent genoms included two chromosomes which were partially homologous. As a consequence, fertility was considerably reduced in contrast to that of the corresponding amphidiploid studied by Karpechenko. Among other intergeneric amphidiploids exhibiting almost completely autosyndetic behavior are the *Triticum-Aegilops* amphidiploids (152, 190, 205) and *Triticum dicoccum* × *Haynaldia villosa* (114). Examples of almost total lack of pairing in F_1 interspecific hybrids and approximately complete autosyndetic conjugation in the amphidiploids are *Galeopsis pubescens* × *G. speciosa* (139), *Nicotiana multivalvis* × *N. suaveolens* (102) and *Triticum turgidum* × *T. villosum* (14).

In many amphidiploids conjugation is almost entirely autosyndetic in spite of the occurrence of some pairing in F_1 meiosis. Usually, loose association is found in such F_1 hybrids, because the chromosomes concerned are not homologous over their entire lengths and few chiasmata are formed. In spite of the amount of structural similarity indicated by their behavior in F_1, they fail to pair in the amphidiploid, due to "differential affinity." The hybrid *Nicotiana glutinosa* × *N. Tabacum* and its amphidiploid follow this pattern (29). In the F_1 an average of 5 bivalents was observed, but in the amphidiploid autosyndesis almost invariably results in 36 pairs. Other amphidiploids of this type are *Digitalis purpurea* × *D. ambigua* (25), and *Quamoclit coccinea* × *Q. pennata* (79).

The prerequisite for the opposite extreme of conjugation—complete allosyndesis in addition to autosyndesis—is extensive structural homology of the two parental genoms, as in closely related

species. In such cases, each chromosome finds a partner in the F_1 diploid hybrid meiosis, barring some genic interference such as the presence of "asynaptic" genes. In the amphidiploid this chromosome homology of the F_1 parents will obviously lead to allosyndesis as well as autosyndesis and thus to the formation of multivalents. Thereafter, distribution to the two poles may be irregular, the mode of chromosome separation frequently resulting in the passing of two identical chromosomes to the same pole. Moreover, crossing-over in allosyndetically synapsed segments introduces further qualitative disparities in chromosome allotment. The consequent gametic variation in chromosome number and constitution usually causes reduction in fertility and inconstancy of the amphidiploid progeny. The amphidiploid *Crepis rubra* × *C. foetida* (159) illustrates this situation.

An exception to the general rule that complete pairing in the diploid hybrid is prophetic of allosyndesis in the corresponding amphidiploid is the situation encountered in the hybrid *Fragaria bracteata* × *F. Helleri* (74). In the diploid the 7 *bracteata* chromosomes paired with the 7 *Helleri* chromosomes. In the amphidiploid MI the two gametic sets of *bracteata* were separated from those of *Helleri* and pairing was purely autosyndetic. Another deviation from the usual correlations of pairing in diploid and tetraploid hybrids was found in *Primula kewensis,* the amphidiploid of *P. floribunda* × *P. verticillata* (147, 207). Although virtually complete pairing occurred in the diploid hybrid, the amphidiploid usually showed bivalents only, but this behavior was occasionally modified by the formation of 1 to 3 quadrivalents.

Chromosome behavior in many amphidiploids places them in the series transitional from autosyndesis alone to the maximum amount of allosyndesis. The amphidiploids *Nicotiana Tabacum* × *N. sylvestris* (171, 172, 198) and *Nicotiana rustica* × *N. paniculata* (118, 180) are good illustrations of this intermediate position, because knowledge of the relationships of the species involved in both hybrids makes it possible to appreciate the basis of allosyndesis. As already noted, 12 chromosomes of the *N. Tabacum* gametic set ($n = 24$) are believed to have originated from a progenitor of *N. sylvestris* ($n = 12$); and similarly 12 chromosomes of *N. rustica* ($n = 24$) were probably derived from an ancestral form of *N. paniculata* ($n = 12$). Both F_1 hybrids show *Drosera*-type pairing, with

approximately 12 pairs and 12 univalents at MI. In the amphidiploid *N. Tabacum* × *N. sylvestris,* Ternovsky (198) reported 33 to 36 units in MI plates, indicating the presence of some multivalents. A higher percentage of multivalents in an amphidiploid from the same species (supplied by Eghis) was found by Rybin (171, 172) ; in this case there were from 28 to 36 units at MI, 28 appearing most frequently. Neither Ternovsky nor Rybin indicated the distribution of chromosomes as quadrivalents, trivalents, bivalents and univalents, but from their data it appears that complete quadrivalent formation between the chromosomes of *sylvestris* origin did not take place. Lammerts' investigation (118) of meiotic cytology in the *N. rustica* × *N. paniculata* amphidiploid revealed 24 to 36 units, with 10 quadrivalents and 16 pairs occurring most frequently. Singleton (180) found in an amphidiploid involving the same species that there were frequently 36 pairs, but more often from 1 to 4 quadrivalents, 1 to 10 pairs, and the remaining chromosomes distributed as univalents. The fact that quadrivalent formation involving all chromosomes of *N. sylvestris* origin did not occur in the first-mentioned amphidiploid, and that all chromosomes descended from a common *N. paniculata* ancestor were rarely conjugated in quadrivalents in the *N. rustica* × *N. paniculata* amphidiploids, may be indicative of extensive evolutionary alteration in the two allied groups of chromosomes of these amphidiploids, and strengthens the contention that multivalent conjugation in these forms should be attributed not to autosyndesis but to allosyndesis. Among the other amphidiploids in which partial allosyndesis has been reported are *Nicotiana glauca* × *N. Langsdorffii* (105), *Dahlia* sp. × *D.* sp. (120) and *Lycopersicum esculentum* × *L. racemigerum* (2).

Lindstrom (130) correlated genetic ratios of auto- and allotetraploids with four methods of synapsis and subsequent types of disjunction, and Skalinska (184) postulated a fifth system of conjugation and disjunction. The first possibility is complete autosyndesis without segregation. The amphidiploids of F_1 *Raphanus sativus* × *Brassica oleracea* (82, 83) and of F_1 *Nicotiana glutinosa* × *N. Tabacum* (29) are of this type. The second is a somewhat hypothetical method involving preferential allosyndesis and an F_2 phenotypic ratio of 15 : 1. Apparently no instance of such behavior has been reported.

Random assortment of four chromosomes is postulated in the third mode. In an amphidiploid in which quadrivalents are formed at prophase, the chromosomes later emerge as bivalents. Random assortment of these chromosomes to gametes leads to a 35 : 1 F_2 phenotypic ratio. This ratio is also obtained from assortment following random pairing of four chromosomes, no quadrivalents being formed (218). Poole (160) reported random assortment of two allelomorphic character pairs, "nodding-erect" buds and "purple-yellow" anther-tube patterns, in the amphidiploid *Crepis rubra* × *C. foetida.* The characters nodding buds and purple anther-tubes were introduced by *C. rubra,* and erect buds and yellow anther-tubes by *C. foetida.* The assumption is that amphidiploid plants with the genetic constitution *PPPP* possessed purple anther-tubes, those with *pppp* yellow tubes, and all those of a heterozygous condition purple-tipped, yellow-bodied anther-tubes. The observed ratio of 2 purple : 40 tipped : 2 yellow paralleled the expected ratio of 1 : 34 : 1 closely enough to be considered significant, since a population of only 44 plants was studied. Interpretation of the inheritance of nodding *versus* erect buds on the basis of random assortment of chromosomes, modified by quantitative interaction, was likewise supported by the breeding evidence. Yarnel (218) has similarly demonstrated random assortment of chromosomes in a study of flower-color inheritance in the amphidiploid *Fragaria bracteata* × *F. vesca.* In this case random pairing was responsible for random assortment.

The fourth type of synapsis and disjunction involves random assortment of eight chromatids in amphidiploids in which allosyndesis and segmental interchange among the eight chromatids takes place. Since the position of any given gene with respect to the insertion region determines the crossing-over value, the maximum expression of crossing-over results in random assortment of the eight genes concerned. Genes borne near the insertion region tend to assort as in method 3, because there is less chance of an interchange separating sister chromatids. The F_2 phenotypic ratio resulting from random assortment of eight chromatids in an amphidiploid is 20.8 : 1. The closer the genes in question are to the insertion region, the more nearly the F_2 ratio approaches 35 : 1. The amphidiploid *Lycopersicum esculentum* × *L. pimpinellifolium* is representative of random chromatid assortment (130, 131).

Gene ratios for four of five chromosomes studied genetically were less than the $35 : 1$ F_2 ratio typical of random chromosome assortment. The genes Yy showed a ratio of $31 : 1$, indicating that these genes lie close to the insertion region; genes Aa must be farther from the insertion region, since their ratio was $28 : 1$; genes Dd showed a ratio of $25 : 1$, indicating still greater distance; and the ratio in the case of genes Rr was $22 : 1$, closely approaching the $21 : 1$ ratio of the completely random assortment of eight chromatids.

Cytogenetic analysis of flower color inheritance in the amphidiploid progeny of *Aquilegia chrysantha × A. flabellata* by Skalinska (184) is of interest because interpretation of chromosome conjugation does not coincide with any of the four modes listed by Lindstrom, effects of allo- and autosyndesis on the segregation of interacting factors are demonstrated, and the progeny provides an illustration of "shift." In this amphidiploid four factors interact in flower-color inheritance—C_1 (anthocyanic base) which is linked to Y (yellow), R (pink anthocyanin) modified to blue by the presence of F. As in Lindstrom's method 3, the assortment of four allosyndetically conjugated chromosomes is involved; but in this case the reversed sequence of quadrivalent and bivalent formation is postulated. Skalinska infers that in early prophase autosyndetic bivalents C_1-C_1 and c_1-c_1 are formed, later in prophase becoming loosely united into a rod-quadrivalent. Anaphase separation at the allosyndetic union causes both chromosomes carrying C_1 to pass to one pole and those with c_1 to the other. Autosyndetic conjugation of chromosomes carrying, respectively, R, F and their recessives, results in the presence of both R and F in all gametes. Thus the phenotypic ratio of the amphidiploid progeny is 3 blue : 1 white. "Shift," the suppression of a parental character to the extent that it is not recovered in the allopolyploid progeny (47, 31, 174), is illustrated by the absence in the amphidiploid progeny of the yellow flowers characteristic of *A. chrysantha*. Factor Y is hypostatic to R; and since C_1, which supplies a base for the expression of R, is linked to Y, and due to the presence of R in all gametes, yellow pigmentation is completely suppressed.

Inconstancy of amphidiploids is caused by four conditions: a) aneuploidy resulting from unequal quantitative distribution of chromosomes to the gametes, b) genetic variation due to unequal

qualitative assortment of the chromosomes to the gametes, although the gametes possess the exact euploid number of chromosomes, c) crossing-over and segregation of cross-over products, and d) chromosome reorganization. In the amphidiploid *Nicotiana glauca* $\times$ *N. Langsdorffii* (105) segregation was probably influenced by all these conditions. The somatic chromosome number of the euploid form was 42, but F_2 to F_6 generations included plants with chromosome numbers ranging from 21 to 52, with corresponding cytogenetic, morphological, physiological and biochemical inequalities. Since plants with 42 chromosomes were dissimilar, unequal qualitative assortment of chromosomes must have taken place. Evidence was cited which indicated that crossing-over between *N. glauca* and *N. Langsdorffii* chromosomes probably occurred. Finally, chromosome reorganization was found in the *N. glauca* satellited chromosome, the long arm of which was reduced in the F_3 amphidiploid, while in an F_4 plant the short arm was significantly elongated.

It appears from the literature on amphidiploids that many, perhaps the majority, are subject to quantitative and qualitative irregularities in chromosome assortment. But among those reported as being constant are *Brassica napus* $\times$ *B. campestris* (52), *Fragaria bracteata* $\times$ *F. Helleri* (74), *Quamoclit coccinea* $\times$ *Q. pennata* (79), *Aesculus hippocastanum* $\times$ *A. pavia* (186), *Pelargonium radula roseum* (179), *Nicotiana glutinosa* $\times$ *N. Tabacum* (29), *Triticum turgidum* $\times$ *T. villosum* (204), *Aegilops ovata* $\times$ *Triticum durum* and *Aegilops ovata* $\times$ *Triticum dicoccoides* (205), and *Erophila verna* $\times$ *E. violacea-petiolata* (215).

The mutation rate in amphidiploids may differ from that of the parental species involved (105), the implication being that this difference is referable to increase in chromosomal alterations and gene mutations in species hybrids. Kostoff (105) postulated that such mutations and chromosome rearrangements are probably responsible for the gradual increase in the fertility of amphidiploids. Dobzhansky (37) suggests that, in the absence of evidence to the contrary, the genes of polyploids mutate just as frequently as in diploids, while Huskins (73) maintains that meiosis in polyploids is productive of more mutations than in diploids and that, although few gene mutations are known, chromosome alterations are common and, if not too severe, are generally viable. Determination of

polyploid mutation rates is rendered difficult by the impossibility of detecting recessive mutants unless they are present in all chromosome sets, for when a recessive mutation occurs in one set only, its phenotypic manifestation is suppressed by normal allelomorphs in other chromosome sets (37). The visible mutation rate should be more rapid in amphidiploids of distantly related parents, since they contain fewer duplicated genes (192). In amphidiploids of closely related parents deleterious mutations may fail to be eliminated by natural selection, due to the presence of the normal allelomorphs (37).

FERTILITY AND CROSSABILITY

In general, a negative correlation exists between the fertility of an F_1 hybrid and that of its corresponding amphidiploid (33, 176, 37). Hybrids of distantly related species are usually sterile, due to minimum chromosome pairing and the resulting chromosomally unbalanced gametes. Their corresponding amphidiploids, in which MI configurations show primarily bivalents, are usually fertile. Among the fertile amphidiploids of sterile or nearly sterile diploid hybrids are *Erophila verna × E. violacea-petiolata* (215), *Raphanus sativus × Brassica oleracea* (83), *Galeopsis pubescens × G. speciosa* (140, 141), *Collinsia bicolor × C. bartsiaefolia* (69), *Triticum Timopheevi × T. monococcum* (101), *Nicotiana suaveolens × N. alata* (111), *Aegilops ovata × Triticum dicoccoides* and *Aegilops ovata × Triticum durum* (205), *Quamoclit coccinea × Q. pennata* (79), *Nicotiana glutinosa × N. Tabacum* (27, 29), *Nicotiana multivalvis × N. suaveolens* (102), *Nicotiana glauca × N. Langsdorffii* (105), *Nicotiana Tabacum × N. otophora* (22), *Mentha aquatica × M. rotundifolia* (146) and *Triticum monococcum × Aegilops uniaristata* (178).

When closely related species enter into the formation of the hybrid, the diploid is likely to be fertile because of complete or nearly complete conjugation. The amphidiploid of such a hybrid frequently shows a reduction in fertility, due to multivalent formation and irregular assortment of the chromosomes to the gametes. The amphidiploids *Crepis rubra × C. foetida* (159, 160) and *Aquilegia chrysantha × A. flabellata* (184) belong in this category.

The foregoing generalizations concerning conjugation in relation to fertility are not without exceptions. In the diploid hybrid *Primula floribunda × P. verticillata* (147), pairing is as complete

as in a pure species, but these plants are sterile; whereas in the amphidiploid, conjugation in bivalents is the usual condition and fertility results. Another exception to the rule is the condition found in *Fragaria bracteata* × *F. Helleri* (74); in the F_1 hybrid pairing is complete and fertility good, yet in the amphidiploid only bivalents occur in meiosis, which leads to high fertility.

Conditions other than gametic chromosome unbalance due to irregular assortment of chromosomes may influence fertility of amphidiploids. Greenleaf (60) found that the amphidiploids *Nicotiana sylvestris* × *N. tomentosa* and *Nicotiana sylvestris* × *N. tomentosiformis,* despite regular meiosis and over 90% good pollen, were completely self-sterile. Amphidiploids of F_1 *Nicotiana sylvestris* × *N. Setchellii* and F_1 *N. glutinosa* × *N. tomentosa* showed somewhat less regular meiosis and were also self-sterile. It was observed that in most cases development of the embryo sac did not proceed beyond the 2- or 4-nucleate stage, and disintegration followed. Greenleaf postulated the presence of complementary sterility genes, carried on not more than two or three chromosomes of either parental genom, which affect embryo-sac development but do not interfere with the viability of the pollen. The possibility of establishing fertile strains by eliminating a few chromosomes carrying the sterility genes was mentioned. Three other amphidiploids obtained by crossing an autotetraploid *N. sylvestris* with three heterozygous autotetraploid varieties of *N. tomentosa* were partially fertile, indicating that the major sterility genes of the previously mentioned sterile amphidiploids are absent in these partially fertile ones. Kostoff (108) reported a fertile *N. sylvestris-tomentosiformis* amphidiploid, developed from the same strains employed by Greenleaf. Since Kostoff's amphidiploids were produced by crossing F_1 *N. sylvestris* × *N. tomentosiformis* with *N. sylvestris* and then crossing the sesquidiploid with *N. tomentosiformis* (unreduced gametes involved in each case), the absence of sterility may have resulted from chromosomal alterations due to gametic variation, and thus the elimination of chromosomes carrying sterility genes.

Sirks (181) adduced six incompatibility factors to explain self- and inter-sterility in plants of *Verbascum phoeniceum,* at that time considered a diploid species. Lawrence (121) showed that a better approximation to observations of incompatibility factor seg-

regation is obtained when *V. phoeniceum* is considered an amphidiploid species. His theory of incompatibility in polyploids is that *"like* factors in pollen and style *positively* inhibit and *unlike* factors *positively* promote pollen tube growth, but the potencies of these two opposite reactions are unequal." Thus a pollen tube carrying factors S_1S_1 is unable to grow in an $S_1S_1S_2S_2$ style; yet S_1S_3 pollen functions normally in the same style. A positive reaction favouring pollen tube growth is set up by the S_3 gene in the pollen, even though S_1 is present in the same male gamete, since the reaction of S_3 with S_1 dominates the reaction of S_1 with S_1. Müntzing (139, 140) analyzed cases of intraspecific sterility in *Galeopsis Tetrahit,* the natural amphidiploid of *G. pubescens* and *G. speciosa,* and concluded that it was due to heterozygosity and lethal recombination gametes and that a difference in viability of male and female gametes obtained.

In the early generations following amphidiploidy, fertility is often reduced, but in later generations an increase in fertility may be achieved through segregation. Thus, in the original amphidiploid *Nicotiana glauca × N. Langsdorffii* (105) approximately two-thirds of the pollen mother cells contained multivalents, univalents, or both, which reduced the number of viable pollen grains to about 51%. Coincident with a decrease in numbers of multivalents and univalents, pollen viability was augmented in the F_2 generation to 59%, in the F_4 to 94%, and in selected plants of the F_6 to 99.5%, with increase in number of seeds per capsule approximately parallel. Although fertility in general increased with successive generations, certain plants with low fertility, and even completely sterile ones, occurred.

Preservation of amphidiploid lines is frequently insured by some degree of incompatibility with the original parents (142, 90). Without this sexual isolation amphidiploids tend to revert to the parental types as a result of backcrossing. Müntzing (142) lists three possible conditions of crossability between polyploids and their parental species:

a) Complete incompatibility, enabling the amphidiploid, if it is viable, to maintain an independent existence. In this category are the artificial *Galeopsis Tetrahit* and its parental species *G. pubescens* and *G. speciosa;* attempts to backcross *G. Tetrahit* to the original parents met with complete failure.

b) Hybrids are formed but are completely sterile. If reciprocal crosses are equally successful, the relative sizes of amphidiploid and parental species populations are not altered. But if crosses succeed more readily when the amphidiploid is used as the female parent, the size of the amphidiploid populations will tend to decrease (27, 142). Backcrosses of the amphidiploid *Digitalis purpurea × D. ambigua* resulted in completely sterile hybrids (23); and the amphidiploid *Nicotiana rustica × N. paniculata* (118) probably falls in this category.

c) Partially or considerably fertile hybrids are formed, from which the parental types are obtained after continued backcrossing. The amphidiploid *Nicotiana digluta* (*N. glutinosa × N. Tabacum*) (27) crossed readily with both *N. glutinosa* and *N. Tabacum* when *digluta* was used as the female parent. The backcross to *N. glutinosa* yielded no progeny, but since a few seeds were produced, there remained the possibility that additional crosses might yield viable ones. From backcrossing *N. digluta* to *N. Tabacum* a highly fertile progeny was obtained. Because of the extensive elimination of *glutinosa* univalents, selfing or further backcrossing would have resulted in a reversion to *N. Tabacum*.

The behaviour of the amphidiploid *Raphanobrassica* in backcrosses (89, 86, 87) was comparable to that of *N. digluta,* although crosses were more difficult to obtain. When pollinated with *Raphanus,* the progeny was slightly fertile. Due to meiotic elimination of cabbage chromosomes, the progeny of the backcross rapidly reverted to *Raphanus.* Similarly, in the backcross to *Brassica,* the seeds obtained produced cabbages, as a result of elimination of radish chromosomes, which the authors believed to have occurred in the first division of the zygote.

Incompatibility between an amphidiploid and its original parents may result from checked pollen tube growth or disturbance in seed development (142). Failure of pollen tubes to pass through the style may be due to a shift in the $2:1$ chromosome number relations of style and pollen tube normally found in self-fertilization or in crosses between races having the same chromosome numbers. In many dicotyledons a proportionately higher chromosome number of the style does not inhibit pollen tube growth, but in reciprocal crosses pollen tube development is checked. This may account for the failure of the crosses *Nicotiana Tabacum* ♀ or *N. gluti-*

nosa ♀ × *N. digluta* ♂, although the reciprocal crosses were successful. However, in the Gramineae, when crosses are made between low ♀ and high ♂ chromosome numbers, pollen tube development is often as good as or better than that of hybrids involving high ♀ × low ♂ numbers (93).

Disturbances in seed development in amphidiploid backcrosses are probably caused by an unbalance due to a change in chromosome number relations of the tissues concerned (142). In self-fertilization and in crosses between species having the same chromosome numbers the ratio of chromosome numbers of embryo, endosperm and the surrounding maternal somatic tissues is 2 : 3 : 2. The union of gametes with different chromosome numbers may alter this ratio and impair the morphological and probably physiological relationships of the resulting tissues and be followed by defective development or complete abortion of seeds.

Incompatibility is not limited to crosses of amphidiploids with the parental races, but also applies to hybridization of amphidiploids with forms closely related to the original parents (90). On the other hand, amphidiploids may be crossed with species with which the parental species do not hybridize. Thus, *Galeopsis Tetrahit* may be crossed to *G. bifida,* with which the parents of *Tetrahit* do not cross (140); and *Raphanobrassica* hybridizes readily with *Brassica carinata* and *Brassica napus,* two species with which it is practically impossible to cross the parental species of *Raphanobrassica* (87).

EVOLUTIONARY SIGNIFICANCE AND DISTRIBUTION OF AMPHIDIPLOIDS

The comparatively recent recognition of amphidiploidy as a naturally occurring and not uncommon phenomenon has necessitated some reëvaluation of concepts of species origins and relationships and thus of evolutionary mechanisms. Increasing importance is being given to polyploidy, especially to allopolyploidy (according to Sansome and Philip [174], it is almost a constant feature of wild polyploids that they are allo- and not autopolyploids) as an instrument in evolution and to the necessity of using cytogenetic evidence in attempts to resolve the phylogeny of plant groups.

Amphidiploidy makes for relationships which are expressed in reticular rather than dendritic fashion—a complex interwoven net-

work rather than a tree or branched chain (5). Although acceptance of the amphidiploidy concept as a factor in evolution is an aid to determining evolutionary patterns, the relationships which amphidiploidy sets up are frequently difficult to unravel.

As already mentioned, Winge (213) first accounted for the arithmetical progression of chromosome numbers in the species of a genus as due to polyploidy. In Fernandes' table containing the chromosome number distribution of 2,413 species, not a single mode occurred at a prime number (50). This fact favors the view that in the evolution of angiosperms, species with chromosome numbers in a multiple series are favored, with respect to either origin or persistence, and emphasizes the far-reaching significance of polyploidy.

Concerning the incidence of polyploidy in angiosperms no exact figures can be cited. However, from evidence compiled by Stebbins (191) concerning the extent of polyploidy in annual, perennial herbaceous, and woody genera, it appears that 71% of the perennial herbaceous genera known cytologically include more than 25% polyploids, whereas only 14% to 24% of the annual genera contain more than 25% polyploids. This distinction in prevalence of polyploidy is explained by Stebbins as due in part to differences in the length of life of annuals and herbaceous perennials; chromosome doubling in a sterile annual hybrid must be accomplished in a single growing season, whereas in perennials opportunities for chromosome doubling are not thus limited by the time factor. Moreover, the acceleration of vegetative development and decrease in reproductive growth commonly associated with polyploidy would be detrimental to polyploid annuals, since a highly developed reproductive phase in annuals is necessary for their survival. Of the woody genera known cytologically, 33% to 36% includes more than 25% polyploids. The cytological stability of woody plants is suggested by Stebbins as a possible explanation of the lower percentage of polyploids in woody plants than in perennial herbs. Interspecific hybrids of several woody genera show regular meiotic behaviour, and therefore amphidiploidy would not be expected in those genera, since the stimulus to chromosome doubling supplied by incompatibility of genoms is lacking.

Some generalizations have been attempted with regard to occurrence of polyploidy and conditions which inhibit or promote chro-

mosome doubling in certain families. Heilborn (67) listed the following conditions which he considered necessary for major incidence of polyploidy: a) somatic doubling or production of unreduced gametes, b) only a slight degree of cell-constancy, c) possibility of self-fertilization, d) capacity for enduring a change from the dioecious condition to hermaphroditism, e) barriers which prevent swamping of the newly established polyploids by crossing with the diploid parents, and f) regularity in meiotic processes. Where one or more of these conditions is lacking, polyploidy rarely occurs or is altogether absent.

In the Cyperaceae three-fourths of each pollen tetrad tend to degenerate, a condition which Heilborn (67) believes to be responsible for the absence of dyad formation in species hybrids and, hence, the failure of allopolyploidy through fusion of unreduced gametes. Aneuploidy, however, is common in certain genera of the Cyperaceae. Heilborn suggests that groups of organisms with abundant chromosome fragmentation are predisposed toward aneuploidy and rarely toward polyploidy; furthermore, that families in which autopolyploidy is common and allopolyploidy rare will often produce aneuploids. On the other hand, in families where multiple chromosome numbers are characteristic and the chromosome numbers constant, allopolyploidy represents the mode of origin of many sub-groups within these families. According to Darlington (33), absence of polyploidy in many groups of flowering plants may result from failure of polyploid species to segregate or mutate toward dwarfness, the deleterious effects of the gigantism frequently associated with chromosome doubling being responsible for the non-survival of such polyploids. He also suggests that infrequency of polyploidy in genera characterized by long chromosomes, as in many genera of the Liliaceae, may be due to abnormal behaviour of those chromosomes when in the polyploid state.

Dissolution of genetic barriers and exchange of genes between genetic systems that are completely isolated from each other in the diploid condition are made possible by the synthesis of polyploid complexes through allopolyploidy between three, four or more species, following the introduction of genes from all the species concerned (192). Complex relationships in *Crepis, Rosa, Rubus, Taraxacum* and certain other genera have resulted from the preservation of numerous allopolyploid and partially polyploid

types of hybrid origin. These series of intermediate types completely obliterate the gaps between species that were once distinct, even between distantly related species (192). In the genus *Crepis* all but one of the 16-chromosome species have been shown to be either amphidiploids or autotetraploids, the 22-chromosome species are products of interspecific hybridization and amphidiploidy, while the higher-numbered species are polyploids derived from them (8). Similarly, the balanced polyploid forms of the genus *Rosa* probably originated through hybridization and chromosome doubling, the unbalanced types later arising from further hybridization followed by apomixis (18).

All amphidiploids involving distinct species deserve at least specific rank and, according to Anderson (5), if the parental stocks are sufficiently distinct, there is no reason why a new genus, new family, or a new order might not originate in this manner. Stebbins (192), however, maintains that although allopolyploidy appears to have given rise sporadically to new genera and perhaps even to families, it is probably not a significant factor in the origin of major plant groups. Furthermore, it is unlikely that polyploid complexes originate new lines of evolution, since, as compared to a group of diploid species, a polyploid complex tends to be a closed system, producing additional species which represent the same genic material in new and different combinations.

Amphidiploids from F_1 hybrids in which meiosis is asyndetic may give rise to monomorphic species, but when gametic sets of the F_1 are relatively equivalent and allosyndesis occurs, and if a series of segregated forms can survive, a polymorphic species is produced. Since inconstant amphidiploids may originate a series of adaptable forms, they frequently afford more suitable material for natural selection than the highly constant amphidiploids (105). Among cultivated plants the value of a naturally occurring or artificially produced amphidiploid may be lessened by the presence of certain morphological or other characters. This condition may sometimes be ameliorated by gene transfer through backcrossing (12).

By contrast with the somewhat restricted distribution of the diploids of a polyploid complex, the polyploids are more widespread —the allopolyploids occupying the extremes of the range, while the autopolyploids are more limited in extent (192). Tetraploids may

extend farther north than diploids (6, 177), may be more concentrated in alpine regions, or may be more widely distributed along the sea coast than diploids. Whereas Hagerup (65) believes that unfavourable environmental conditions may induce polyploidy and that desert plants, for example, will show a high degree of polyploidy, Sax (177) maintains that although polyploidy might be induced by temperature extremes as the diploid races extend their ranges, it is probable that the polyploids originated prior to such extension and survive because of greater hardiness. According to Stebbins (192), the acquisition by allopolyploids of advantageous new combinations of physiological characters permitting more ready adaptation to localities unsuitable for the diploid or autopolyploid species may explain the extension of their ranges. Whereas diploids tend to inhabit older, more stable habitats, polyploids are dominant in areas more recently opened to plant occupation or in regions where climatic or other environmental conditions have undergone alteration. Since polyploid members of a complex are more numerous and widespread than diploids, it is to be expected that as a polyploid complex ages and as conditions become more unfavourable for its survival, extinction of the diploids occurs first. Thus, an old polyploid complex may come to consist entirely of polyploids. Gradual extinction of polyploids reduces the complex in its last stages to a monotypic or ditypic genus without close relatives. In regard to major lines of evolution polyploidy is more important in preserving relics of old genera and families than in giving rise to new ones (192).

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<div align="center">REPORTED AMPHIDIPLOIDS</div>

Aegilops caudata × *A. umbellulata* (178)

Aegilops caudata × *Triticum dicoccum* (152)

Aegilops longissima × *Triticum durum* (190)

Aegilops ovata × *Triticum dicoccoides* (93, 95, 205)

Aegilops ovata × *Triticum durum* (205)

Aegilops ovata × *Triticum turgidum* (155)

Aegilops spelioides × *A. umbellulata* (94, 178)

Aegilops triuncialis × *Triticum dicoccoides* (190)

Aegilops triuncialis × *Triticum dicoccum* (152, 190)

Aegilops triuncialis × *Triticum durum* (153)

Aegilops ventricosa × *Triticum durum* (189)

Aegilops triuncialis × *Triticum polonicum* (190)

Aesculus hippocastanum × *A. pavia* (51, 186, 206)

Anemone sylvestris × *A. magellanica* (76)

Aquilegia chrysantha × *A. flabellata* (182–185)

Betula verrucosa × *B. pubescens* (68)

Berberis Darwinii × *B. empetrifolia* (201)

Brassica campestris × *B. oleracea* (145)

✓ *Brassica campestris* × *B. nigra* (145)

Brassica campestris × *B. oleracea* or *B. alboglabra* (145)

Brassica chinensis × *Raphanus sativus* (194)

Brassica napus × *B. campestris* (52)

Brassica nigra × *B. oleracea* or *B. alboglabra* (145)

Brassica oleracea × *B. carinata* (85)

Brassica oleracea × *B. chinensis* (88)

Collinsia bicolor × *C. bartsiaefolia* (69)

Crepis capillaris × *C. dioscoridis* (9)

Crepis capillaris × *C. tectorum* (70, 9)

Crepis rubra × *C. foetida* (9, 159, 160)

Cucurbita maxima × *C. moschata* (21)

Dahlia sp. (ivory-magenta-purple group) × *D.* sp. (yellow-orange-scarlet group) (120, 122)

Delphinium sp. × *D.* sp. = *D. Belladonna* (123)

Delphinium sp. × *D.* sp. = *D. Lamartinii* (123)

Dianthus sinensis × *D. Knappii* (7)

Digitalis purpurea × *D. ambigua* (25, 23, 24)

Erophila verna × *E. violacea-petiolata* (215)

Euchlaena perennis × *Zea Mays* (46, 30)

Festuca arundiaceae × *F. gigantea* (148)

Fragaria bracteata × *F. Helleri* (74, 75)

Fragaria bracteata × *F. vesca* (218)

Fragaria vesca × *F. chiloensis* (217)

Galeopsis pubescens × *G. speciosa* (138–141)

Galium mollugo × *G. verum* (48)

Gossypium arboreum × *G. thurberi* (12)

Gossypium (Asiatic) × *G.* (wild American) = cultivated American (211*a*, 186*a*)

Gossypium barbadense × *G. herbaceum* (12)

Gossypium herbaceum × *G. anomalum* (219)

Gossypium hirsutum × *G. arboreum* (12, 219)

Gossypium hirsutum × *G. armourianum* (219)

Gossypium hirsutum × *G. barbadense* (12, 91)

Gossypium hirsutum × *G. harknessii* (12)

Gossypium hirsutum × *G. herbaceum* (12)

Gossypium hirsutum × *G. Sturtii* (219)

Gossypium hirsutum × *G. Stocksii* (219)

Hyoscyamus niger × *H. albus* (64, 63)

Hibiscus esculentus × *H. Manihot* (199)

Iris virginica × *I. setosa* (3)

Lycopersicum esculentum × *L. racemigerum* (2)

Lycopersicum pimpinellifolium × *L. esculentum* (129, 131)

Medicago falcata × *M. sativa* (202)

Mentha aquatica × *M. rotundifolia* (146)

Narcissus pseudonarcissus × *N. poeticus* (136)

Nicotiana Benthamiana × *N. Debneyi* (22)

Nicotiana Bigelovii × *N. suaveolens* (54)

Nicotiana excelsior × *N. velutina* (106)

Nicotiana glauca × *N. Langsdorffii* (97, 99, 105)

Nicotiana glutinosa × *N. glauca* (187)

Nicotiana glutinosa × *N. sylvestris* (58, 21)

Nicotiana glutinosa × *N. Tabacum* (29, 27, 195–197, 135)

Nicotiana glutinosa × *N. tomentosa* (55, 45, 58–61)

Nicotiana glutinosa × *N. tomentosiformis* (58)

Nicotiana maritima × *N. plumbaginifolia* (22)

Nicotiana multivalvis (*N. Bigelovii* var. *multivalvis*) × *N. suaveolens* (102)

Nicotiana paniculata × *N. solanifolia* (22)

Nicotiana rustica × N. glauca (100)
Nicotiana rustica × N. paniculata (117, 118, 180, 98, 99, 22)
Nicotiana rustica × N. Tabacum (103, 161, 187)
Nicotiana Setchellii × N. otophora (22)
Nicotiana suaveolens × N. alata (106, 111)
Nicotiana suaveolens × N. Sanderae (111)
Nicotiana sylvestris × N. Setchellii (60, 61)
Nicotiana sylvestris × N. tomentosa (58–61)
Nicotiana sylvestris × N. tomentosiformis (97, 104, 111, 58–61)
Nicotiana Tabacum × N. glauca (137, 195, 197, 161, 187, 188, 175)
Nicotiana Tabacum × N. glutinosa (21, 20, 210)
Nicotiana Tabacum × N. otophora (22)
Nicotiana Tabacum × N. rustica (41, 170)
Nicotiana Tabacum × N. sylvestris (171, 42, 187, 188, 198, 10)
Nicotiana Tabacum × N. tomentosa (97)
Nicotiana velutina × N. rotundifolia (22)
Nicotiana velutina × N. suaveolens (22)
Ocimum canum × O. gratissimum (119)
Pelargonium sp. × *P.* sp. (179)
Pentstemon laetus × P. azureus (26)
Phleum · pratense × P. alpinum (62, 143)
Primula · Bulleyana × P. Beesiana (163)
Primula · floribunda × P. verticillata (36, 154, 147, 207)
Prunus spinosa × P. divaricata (173, 32)
Quamoclit · angulata × Q. pennata (79)

Raphanus sativus × Brassica oleracea (80, 81, 83, 164, 71)
Rosa pimpinellifolia × R. tomentosa (19, 18)
Rubus idaeus × R. caesius (168)
Rubus strigosus × R. rusticanus (31)
Salix viminalis × S. caprea (149, 66)
Saxifraga adscendens × S. tridactylites (39)
Saxifraga rosacea × S. granulata (133, 212, 158)
Solanum melongenum × S. tamago (150)
Solanum nigrum × S. luteum (77)
Spartina alterniflora × S. stricta (72, 78)
Tradescantia subaspera × T. canaliculata (4)
Triticum dicoccoides × Aegilops ovata (95, 93)
Triticum dicoccum × Agropyron glaucum (157)
Triticum dicoccum × Haynaldia villosa (114)
Triticum durum × T. monococcum (200)
Triticum durum × Secale montanum (107)
Triticum monococcum × T. persicum (92)
Triticum monococcum × Aegilops uniaristata (178)
Triticum Timopheevi × T. monococcum (101)
Triticum turgidum × T. villosum (204, 14, 15)
Triticum vulgare × Agropyron glaucum (156)
Triticum vulgare ×Secale cereale (134, 203, 127, 124, 38, 208, 16)
Triticum vulgare × T. compactum (38)
Vicia macrocarpa × V. sativa (193)
Verbascum sp. × *V.* sp. = *V. phoeniceum* (121)

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THE CYTONUCLEAR RATIO

VIVIAN V. TROMBETTA[1]

Smith College

A complete study of size relationships of nucleus to cytoplasm would necessarily be a broad one and should include not only the problem of relationships which exist at maturity as well as those changes which occur during growth and differentiation, but also the physiological problems involved. Such a complete study is beyond the scope of the present paper which will deal chiefly with the question of regularity of cell-nuclear size relationship in growth and maturity; physiological inferences will be mentioned but not treated exhaustively.

Much work has been done on the problem of the nucleocytoplasmic size relationship in plant and animal cells, but although the literature is very extensive, the results remain confusing and often contradictory. Such important phases in the life history of organisms as cell division, senescence and rejuvenescence have been explained on the basis of changes in the relative size of nucleus and cytoplasm, but investigators differ in their opinion as to the relative importance of cell size and nuclear size and as to the nature of their relation to each other. It is the purpose of the present paper to assemble as much as possible of the literature bearing on this topic of nuclear and cell size relationships in plants, and to show to what extent these points of view can be reconciled.

Some workers have believed that the relationship between nucleus and cell is always constant, while others maintain that no such constancy exists but rather wide variability in the relative size of cell and nucleus. These differences have been correlated with such activities as division and differentiation. Metabolic changes in a cell are associated with the interchange of material between nucleus and cytoplasm, and any exchange of material between two such structures may be expected to cause changes in their size relationships. Such changes may well disturb the internal metabolic bal-

ance, and the resulting imbalance may stimulate such activities as division.

Perhaps the primary and most widely recurrent problems in the field are the two related questions: first, of any constancy of the relationship found either universally, or for a species, or even for a given tissue; and secondly, the much wider and more inferential series of questions of the significance of any constancy which may be found. Such questions of significance are: is the nucleus or cytoplasm the more effective agent in control of constancy? what is the mechanism of possible control? what is the physiological significance for cell metabolism of the relationship found? what are the implications of this ratio as to mechanisms of chromosome action and polyploidy? how valid is a relationship of this sort for comparison of different species, different ages of cells, or differentiated cell types within an organism? and what is the possible relationship of size ratio to the mechanism of cell division?

Conflict has been introduced into the field partly because many broad generalizations have been based on restricted and specialized material. Equally confusing to any attempt at coordinating the results has been the difference in methods of measurement, statistical treatment and terminology. The term "size relationship" is here purposely used loosely to cover the wide variety of material accumulated by different methods. The primary measurements, usually in diameters, have often been converted into figures for surface area and for volume. This has been done with the help of formulas and assumptions as to approximate cell form; and too often without the use of constants to keep the figures on any scale of recognized units. The ratios, graphs or other comparisons derived by these methods have been variously expressed to fit the type of material, or the author's special interests. In some cases volume relations have been used, in others surface relations; in some other instances more significant correlation has been found between the surface area of one structure and the volume of another. In dealing with different types of material, problems have been raised as to the physiological equivalence of certain measurements: is it justifiable, in plant cells, to compare total cell volume with nuclear volume both in young meristematic cells and in mature highly vacuolated cells? A similar question would apply to animal cells with large amounts of yolk, and these questions

have usually been ignored, either carelessly or because of the difficulty of differential measurement of "active" cytoplasm.

The terminology, too, has been confused. Some authors have used *cell* volume, while others have used *cytoplasm* (cell minus nucleus) for comparison with nuclear volume. Some authors have wished to compare *active* cytoplasm (*i.e.,* cytoplasm minus vacuoles, yolk, *etc.*) with nucleus, but because of difficulties of measurement their discussions or results are not expressed in quantitative terms. Some have expressed their figures in a nucleocytoplasmic ratio (nuclear volume/cytoplasmic volume, or K/P, or kernplasma ratio of Hertwig); and others have used the same names to apply to the ratio of nuclear volume to total *cell* volume, or even to the reciprocals of either of these ratios. To be sure, any such expressions will be measures of the same kind of function, and the form of ratio used is a matter either of convenience or of adjustment to the needs of the data. Whether one uses *cell* volume or *cytoplasmic* volume, however, for comparison with nucleus, may make a decided difference in the possibility of finding order in the results. The use of one figure or its reciprocal would seem to be a mere matter of convenience were it not so confusing when one author speaks of the nucleocytoplasmic ratio increasing under given circumstances, and has a meaning the opposite of that of another author making a similar statement.

To avoid confusion in this paper, all results or conclusions cited are converted into ratios either of total *cell* volume to nuclear volume (cytonuclear ratio of Sinnott and Trombetta, 1936; C/N), or of *cytoplasmic* volume to nuclear volume (Cp/N). If the vacuole is to be regarded as a part of the cytoplasm, this term would, of course, express an inverse measure of the same function as nucleocytoplasmic ratio. In view of the sharp distinction between vacuole and cytoplasm in most mature plant cells, however, the term cytonuclear seems the less ambiguous. Thus the comparison of trends will be made less confusing, for the Cp/N and C/N ratios are indices of very similar functions. Any ratios dealing with diameters or surfaces will be specified and will be expressed in the same order; *i.e.,* with the cell measurement as numerator.

Among students of the quantitative relationship of nucleus and cytoplasm, there are at present two divergent views. One group of investigators believes that very constant relationships have been

found to exist. Sachs (1892, 1893) and Strasburger (1893) were among the first to note the relative minuteness of meristematic cells and concluded that the working-sphere of the nucleus must be a very restricted one. Strasburger found that in meristems of a considerable number of different plants the *average diameter* of the cells and of the nuclei varies from 5 µ and 3 µ, respectively, in some plants to 24 µ and 16 µ in others, giving in each case a diameter ratio of about 3 : 2. He found this ratio in various plants but it should be noted that his methods failed to take account of the differences normally existing in a single meristem. His figures represented the average of many cell diameters and of many nuclear diameters, and therefore failed to record the differences in ratio which are found between cells of different sizes in a given meristem. Furthermore, it should be noted that even if his figures had been applicable to individual cells, a constant *diameter* ratio of 3 : 2 would scarcely have been an index of constant relation in either of the two possible functionally significant size variables. In respect to either protoplasmic mass (volume) or active surface, such a diameter ratio would represent quite different ratios for different sizes of cells. A relation between cell diameter and nuclear diameter was also found in the yeasts *Endomyces fibuliger* and *Saccharomyces capsularis* by Guillermond (1909) and Henneberg (1915).

Gerassimow (1902) also deduced a constancy of size relation from his studies of nuclei and cells in normal *Spirogyra* as compared with a giant (polyploid) race. This latter he artificially induced by using cold and ether to delay cell division relative to nuclear division. In the two races the ratio of nuclear volumes (1 : 1.94) was not similar to that for cell volumes (1 : 2.88) but was closer to that for cell surface (1 : 2) ; and closest of all to that for lateral surface, without the physiologically neutral end walls (1 : 1.91). The cell to nuclear *volume* ratios for these two races of *Spirogyra* are 544 : 1 and 808 : 1, respectively ; but the nuclear volume to active cell *surface* ratio in each case is 31 : 1. It should be noted that this "constancy" is of a very different sort from that postulated by Strasburger.

There is another group of investigators, however, who believe that the cytonuclear ratio is not constant and self-regulatory, but rather variable. In cells of the cambium of the pine, Bailey (1920)

found that his observations did not support Strasburger's conclusions in regard to the constancy of "specific" cell sizes and nuclear sizes in plant meristems. Bailey worked with ratios for individual cells instead of those for averages, and found the volume ratio of cell to nucleus to vary from 12:1 in ray cell initials to 286:1 in tracheid initials. "It is evident, accordingly, that there is a very much greater variability in the size of meristematic cells in plants than was suspected by Sachs and Strasburger, and that in elements of this type the nucleus may extend its energizing influence to a distance of several thousand instead of a few micra. . . . Nor do these initials contain abnormally elongated giant nuclei. . . . Each initial contains a single nucleus which is centrally located and retains this position during the processes of growth and cytokinesis." Thus by using cambium, in which the differentiation of cell types was more obvious than in the root or stem tip meristem with which Strasburger worked, Bailey avoided the pitfall of averaging data for heterogeneous material, and was led to establish the characteristic differences between cytonuclear ratios of different cell types. Other botanists have reported similar variability. Ensign (1919) found the ratio varying from 2.9:1 to 10:1 in cells of the root tip of *Citrus*. Benedict (1915), Klieneberger (1918) and Tischler (1924) report similar variability.

A number of zoologists indicate that the Cp/N ratio (*i.e.,* cytoplasmic volume to nuclear volume) is not constant and self-regulating; and some of them have shown trends of variation in the ratio which have been interpreted as lending support to one or another hypothesis as to functional relation. Koehler (1912) found a marked increase in the total volume of the nuclei in the egg of the sea urchin *Strongylocentrotus,* up to about the ninth cleavage, whereas the volume of the cytoplasm remained unchanged. Thus, during this period the Cp/N ratio dropped to about one-tenth of its original value. Essentially the same principle applies to the eggs of annelids and gasteropod mollusks, as described by Conklin (1912); that is, the total nuclear volume increases largely at a time when the egg is still dependent on its yolk reserves and when there is no essential increase in the sum of the cell sizes. Though total nuclear volume increases, the average nuclear size is progressively decreasing during cleavage. The presence and unequal distribution of the yolk in these eggs, for instance, in that of *Crepidula,*

raises two questions not sharply focussed in the case of the sea urchin egg in which the small amount of yolk is fairly evenly distributed. First of all, this very marked increase of nuclear volume relative to *total* cell volume is obviously much greater than the increase of nuclear volume relative to active cytoplasm, for the latter is at the same time increasing at the expense of the yolk. But almost surely, even on this basis, the Cp/N ratio is decreasing during cleavage. Secondly, in *Crepidula* the unequal distribution of cytoplasm and yolk is a major factor in producing some very unequal cleavages, and the resulting nucleocytoplasmic ratios show a different kind of variability besides that resulting from rapid nuclear growth. The two equal daughter nuclei resulting from any one cleavage very rapidly adjust their sizes to a rough proportionality with the sizes of their cell bodies. It was shown experimentally that when cleavage was distorted to give two equal cells with unequal proportion of yolk, the nuclei were of different sizes. Their adjustment is more nearly to the volume of the active cytoplasm than to that of the cell as a whole. In cells without yolk the volume ratio of cell to nucleus varies from 15:1 to 9:1 while in yolky cells it varies from 90:1 to 35:1. So in the different blastomeres of the egg there is no constant Cp/N ratio, yet the variability is not chaotic or random, for homologous blastomeres of embryos at the same stage have equivalent ratios. The ratios then would seem to express some function of the factors mentioned above (nuclear growth and yolk distribution) perhaps together with a factor of specific differentiation between the cells. It should be noted further that aside from the factor of continued total nuclear growth, the other nuclear differences are transitory, being adjustments of the resting nucleus to local conditions, for at division, all nuclear plates of the same generation are of about the same size though the protoplasmic masses of their cells vary widely.

The above studies on decreasing Cp/N during cleavage (and the principle probably applies to cleavage in most animal eggs) are complemented by studies of Teissier (1927) on oogonial growth in the coelenterate *Hydractinia*. Here he showed that the dimensions of the nucleus increase *less* rapidly than the dimensions of the cell, but that the relative rates of change of volume of nucleus and cell are constant.

The method of analyzing data has caused some of the difficulty

encountered in interpreting cytonuclear studies. For this reason a
new approach to the problem of cell and nuclear size relationships
was suggested by Sinnott and Trombetta (1936)—a method simi-
lar to that proposed by Huxley (1932) for a study of heterogonic
(allometric; Huxley and Teissier, 1936) growth. The logarithms
of nuclear volume are plotted on the y axis and those of cell vol-
umes on the x axis. For instance, data for the growing point of
Cucurbita Pepo were plotted logarithmically, with the average of
many nuclear volumes for each successive cell-volume class. The
points fall into a rather narrow band which may be represented by
a straight line of slope .38, and this relation holds for a range from
426 to 174,800 cubic micra of cell volume. In this whole growth
range the cell volume increases faster than the nuclear volume so
that the cytonuclear ratio increases. The straightness of the line
indicates a constancy of *relative* rate of change of the two variables,
and the slope of .38 is characteristic of the relationship for the
growing point in this species. So, although the arithmetic value
of the ratio may change progressively in such cases, a regular and
characteristic relation between sizes of cells and nuclei may be de-
termined. In many cases the value of the slope constant approached
.67, indicating that the nucleus is growing, on the logarithmic scale,
about two-thirds as rapidly as the cell. Since the surface of a cell
increases as the square of its linear dimensions and its volume as
the cube, and the logarithmic rate of surface increase is thus two-
thirds that of volume increase, this result would indicate that the
volume of the nucleus is keeping pace with the *surface* of the cell
(or perhaps with the volume of active cytoplasm in vacuolated plant
cells, provided the cytoplasmic film is of the same thickness through-
out the period of growth measured).

A study of a variety of material, but chiefly stem tips and root
tips of a number of representative families of the flowering plants,
leaves of *Elodea* and stem hairs of the tomato (Trombetta, 1939),
showed that there is a good deal of variation in the character of
this cytonuclear relationship. Where differences in cell size appear
at about the same distance from the meristem (*i.e.*, where there is
a differentiation in cell size independent of the growth series),
again it is found that the difference in nuclear size is less than that
of cell size. Thus the actual cytonuclear ratio differs, being greater
in the larger cells. Again a constant relationship was found when

the data were plotted logarithmically, indicating a physiological interdependence of sizes even between these divergent cell types. In root meristems the nuclear volume often increased about two-thirds as fast as the cell volume, thus keeping pace with the cell surface. In stem meristems, the slope constants of nucleus to cell are lower than in the root—also the cytonuclear ratio of the stem is greater. In later development, where division has ceased but the cells are enlarging greatly, three distinct types of cytonuclear behavior were found. In one type, the relationship of nucleus to cell found in the meristem is maintained throughout the active period of enlargement. In the other two types, this relationship is greatly modified, and the relationship of nucleus to cell which occurs in the meristem is not maintained throughout the period of enlargement.

Whaley (1939) showed that this allometric relationship of cell size to nuclear size is maintained in the apical meristems of tomato plants, although the course of development is, by repeated divisions, from larger to smaller cells, rather than *vice versa*.

If the data of a number of investigators are interpreted on the basis of this allometric method of analysis, it will be seen that a number of conflicting views can be reconciled. Trombetta (1939) pointed out that when studied by this method the conception of the relationship of nucleus to cell, as commonly found in the literature, evidently requires certain modifications. The constancy which exists in the size relations of nucleus and cell is not between absolute values but rather between the *relative rates of change* of these structures during growth or differentiation. Thus the two conflicting views as to the cytonuclear relationship—that it is a constant and physiologically important one, or that it is extremely variable —may be reconciled. Strasburger maintained that a constant size relation of nucleus to cell exists in the meristems of the higher plants. Obviously, however, it appears that such constancy as occurs is not one of absolute values, for the cytonuclear ratios have been shown by Bailey, Conklin and others to be extremely variable when measured in absolute terms. It would appear to be rather a constancy in relative rates of change. When volumes of meristematic cells and nuclei of a given species are compared, it is evident that there are marked differences in absolute cytonuclear values between large and small cells, but when they are plotted logarithmically, they tend to fall along a straight line, showing a constancy of

relationship. Large meristematic cells have relatively smaller nuclei in proportion to the size of the cell than small ones do. It is evident that the working-sphere of the nucleus is not a restricted one, but rather wide, as Bailey and Conklin have already pointed out.

Another view of the cytonuclear ratio regards it as extremely variable and unordered. The extreme form of this view must also be modified, for the variability is not random but in many cases follows a definite pattern with the result that a regularity does exist. Bailey found that the ratio of cell size to nuclear size in the elongated initials of the cambium of the pine varies greatly between large and small cells. If the method used by Sinnott and Trombetta (1936) is applied to his data, however, a relationship is found. Although the cytonuclear ratio stated in absolute terms changes markedly between one type of cambial initial and another, and between cambial cells in young pines and old ones, the relative rate of change for all of these is essentially constant. Morgulis (1911) reports a wide variability in the ratio in liver and pancreas cells of the salamander, depending upon changes in the food supply. When the volumes of cells and nuclei reported by him are plotted logarithmically, however, the relative rate of change of nuclear and cell size is found to remain strikingly constant in spite of the fact that the absolute sizes of nuclei and cells decrease markedly during starvation and increase again when the animals are given food. Thus in many cases where the cytonuclear ratio has been reported as variable, a more precise analysis by the method described will show that a constant relationship exists, although it is between rates of change rather than absolute values.

SENESCENCE

The cytonuclear ratio has often been thought to be important in the process of senescence. Minot (1908) maintained that the cause of senescence is the increase of the *cytoplasm* and its products at a rate which far exceeds that of the nucleus. According to his view, the egg at the beginning of development is in a senile condition in which "there is an excessive amount of protoplasm in proportion to the nucleus, and in order to get anything which is young a process of rejuvenation is necessary. . . . During the segmentation of the ovum the condition of things has been reversed so far as the pro-

portions of nucleus and protoplasm are concerned. We have nucleus produced, so to speak, to excess. The nuclear substance is increased during the first phase of development. . . . Rejuvenation depends on the increase of the nuclei. Senescence depends on the increase of the protoplasm and on the differentiation of the cells." Hertwig (1903), on the contrary, maintained that physiological depression, senescence and natural death are associated with an increase in the relative size of the *nucleus*. His primary work was with Protozoa, and the generalization which he derived from this somewhat specialized material has not been found applicable in most Metazoa and plants. In this discussion it is thought better to ignore the problems raised by macro- and micronuclei, and by the complex nuclear behavior associated with conjugation in the Infusoria. The opposite interpretations by Hertwig and Minot of the cytonuclear correlatives of senescence may have depended on generalizations from material which was not comparable.

Conklin (1912) points out that both authors agree on the fact of enormous nuclear growth relative to cytoplasm during segmentation of the egg. Conklin's own work with *Crepidula* showed increase of the nuclear mass, of the order of 10% for each cleavage up to about the 32-cell stage, and not more than 1% later—rather less than the enormous increase described by others, but still an increase. He emphasized further the changes in nuclear size at each stage, such that during the 4-cell stage the Cp/N ratio changed during interphase swelling of the nucleus from a minimal ratio of 7:1 to a maximal ratio of 204:1. And again, different blastomeres of the same generation showed variation in Cp/N from 1 to 14. These large variations led him to value lightly the significance of such ratios as a measure of senescence. Conklin agrees with Minot and Hertwig in the idea that senescence is associated with accumulation in the cell of the products of metabolism and differentiation and that rejuvenation consists in a return to a condition in which these products are largely eliminated. He does not, however, agree with their assumption that changes in the nucleocytoplasmic ratio are the causes of these phenomena. His opinion was close to that of Child (1915) in whose view senescence seems to be associated with a decrease, rejuvenescence with an increase of *metabolism*.

Minot's theory has found some support in the work of Hartmann

(1919*a*) on *Cladocera*. Meyer (1917) tried to measure the volume of the cytoplasm in the palisade cells of *Tropaeolum* in an attempt to find whether the cytonuclear ratio changes with age, and discovered that the ratio was greater in an old yellow leaf than in a young green one. In a study of senescence in the cells of the potato plant, Lutman (1925) found that senescence is accompanied by an increase in cell size without corresponding increase in nuclear size. Trombetta (1939) showed that Minot's theory of senescence found some support from results reported for tomato hairs. Between tomato plants 28 days old and mature ones, the size of the nucleus for any given cell size decreased considerably, with a resulting increase in the cytonuclear ratio.

<div align="center">PHYSIOLOGICAL FACTORS</div>

A number of physiological factors have been found to affect the cytonuclear ratio. Hartmann (1919*b*) studied the effect of temperatures of 11.5° to 42° C on cells of the plerome of *Zea Mays* and *Phaseolus coccineus,* and of the dermatogen of *Pisum sativum*. He found that the cytonuclear ratio is directly proportional to temperature over some of this range. In the large vessel cells of the plerome of *Zea Mays* it varied from 12 : 1 at 11.5° C to 19 : 1 at 31° C. Above this, the temperature effect was doubtful. Boring (1909), on the other hand, found that the size and number of nuclei of *Ascaris* embryos are the same over the temperature range of 18°–37° C. Erdmann (1908) studied the effect of temperature on sea urchin embryos developing at 10° C, 15° C and 20° C. Nuclear size and cell size at a given cleavage stage were found to be inversely proportional to temperature, but the cytonuclear ratios derived from her figures show no consistent temperature effect. At some stages the ratio appears to increase with temperature, at others to decrease or fluctuate. Probably the effect, if any, is below the variability of measurement or sampling. Marcus' work (1906) on sea urchins shows a definite rise and fall of the ratio with a rise and fall of temperature.

Such physiological changes as occur in a cell as the result of temperature changes serve, in some unknown way, to bring about changes in the size relationship of nucleus and cytoplasm. That such changes should differ markedly in their expression in different material offers a real problem. In some forms the cytonuclear ratio

is directly proportional to temperature; in others there appears to be an inverse proportionality. Probably innate differences in the material, as the ability of the *Ascaris* egg to live easily under conditions where it has to undergo a difference of perhaps 20° C within 24 hours, or the much slower penetrability of the egg shell of *Ascaris,* or differences in turgor and osmotic pressure in the cells may account, in part, for the differences in the results. In any case it seems unlikely that any conclusions on which predictions may be based can be deduced from such a study of the temperature effect on cytonuclear ratios.

A lowering of the cytonuclear ratio was induced by X-ray treatment of the leaves of *Nicotiana* (Goodspeed, 1929). In this case the effect was on the cytoplasm which increased considerably in volume while the nucleus showed little change in size.

It is well known that in wound reactions involving dedifferentiation and meristematization of mature tissue, the rapid subdivision of large cells is accompanied by rapid nuclear growth. The implied decrease in cytonuclear ratio has, however, not been expressed in quantitative form except by Riker (1927) in wound tissue of the tomato, and Verplanche (1931) in meristem tissue of the potato affected by the fungus "spindle tuber." The measurements of both these authors confirm the hypothesis.

ACTIVE MASS

In the preceding discussion, the cytonuclear ratios have been derived from measurements of volume, or sometimes of surface area; and speculations have been made as to the physiological significance of the relations between these volumes or areas. Some authors, however, in looking for physiological realities behind the raw size data have thought that other measures might have greater significance than mere volume.

In the nucleus, for instance, since the chromatin is thought to be an essential agent of nuclear metabolism, its mass has been sought as an index to degree of function. Unfortunately it is not practicable to measure the mass of chromatin accurately except at metaphase in some species. Some authors have used the customary measurements of nuclear volume, and have assumed or hoped that these would serve as index to the active mass of chromatin, and that the latter was the real functional correlative of cell size

(Hegner, 1924, in *Arcella*). Boveri (1905) and others have used chromosome number, without consideration of their individual sizes, as an index of active nuclear mass.

In the same way, the active mass has been assumed to be less than the total volume of the cytoplasm. In their considerations of cytonuclear ratios some authors have tried to measure cytoplasmic volume without the vacuole (Meyer, 1917) in palisade cells of *Tropaeolum*. Sinnott and Trombetta (1936) have suggested that where their data show cell surface increasing with nuclear volume, there may really be a volume relation between the active cytoplasm (without vacuole) and the nucleus. Other authors make allowance for clearly defined zones of heavily-yolked and more or less inert cytoplasm (Conklin, 1912, in *Crepidula* eggs).

In seeking valid measures of active mass where sizes are of doubtful significance, the idea of chemical analysis has occurred to various people. Palladin (1896), in plant tissue, tried to dissociate the "active" proteins, *i.e.,* those of nucleus and plastids, from the other proteins by digestion with pig gastric juice. More modern interpretations of the chemical cytonuclear ratio have used as index of nuclear constituents either nucleic acid, guanidine, or purine nitrogen, measured by various techniques, for comparison with total protein or total nitrogen of the cell (tissue). Using ratios of such indices, Lebreton and Schaeffer (1923) have found in developing chick, pig and mouse embryos, an increase in the chemical C/N ratio roughly following the trend indicated by Minot (1908) by size measurements. Likewise, Kahn (1925) showed a decrease in chemical C/N (total N/ purine N) during starvation in rabbits' muscle comparable to the decreasing size ratios found by Morgulis (1911) on starving salamanders.

These chemical indices, then, may be used instead of size ratios, and will give roughly comparable results. They may be obtained on whole embryos (average for all the cells) or for tissues with irregular cell shapes where size measurements are not feasible. But even when the chemical C/N ratios have given clear results on animals, we have no evidence beyond assumption that they follow the prime physiological functions any more closely than do the comparable size ratios, or that chemical measurements are indices of truly active mass. Furthermore, the chemical measures have not yet been widely applied to plant tissues.

CELL DIVISION

The cytonuclear ratio has often been thought to be important in cell division, the ratio having been suggested as a factor in initiating the process (Godlewski, 1910). Strasburger (1893) suggested that cell division occurred when the ratio of the cell body to the nucleus increased beyond a certain point, which might be regarded as marking the limit of the "working sphere" of the nucleus; with the division of the cell the normal ratio was once more restored. Hertwig (1908) explained nuclear and cell division by his concept of "Kernplasma-Spannung," a "tension" favoring nuclear growth, created by an increasing disproportion between nuclear volume and cell volume as the cell enlarges. The intervals between successive mitoses were divided by Hertwig into two periods: a) a period of "functional growth," during which the cytoplasm grows more rapidly than the nucleus, leading to an abnormal kernplasma ratio, and b) a period of "division growth," during which the normal K/P ratio (the "Kernplasma-norm") is regained by rapid growth of the nucleus, and at the end of which cell division occurs. The end of the period of functional growth when the K/P ratio is abnormal is the moment of "nucleocytoplasmic tension." Popoff (1908) has found in the protozoan *Frontonia* that immediately after cell division there is a diminution of the nucleus, which is then followed by a slow relative growth ("functional growth" of Hertwig), and this by a much more rapid growth of the nucleus preceding division ("division growth" of Hertwig). Division and the plane of division are determined at the moment of nucleocytoplasmic tension—*e.g.*, if pieces of cytoplasm were removed during the period of functional growth, the cytoplasm continued to increase until the nucleocytoplasmic norm was established, but if they were cut away during the period of division-growth, division occurred without the regaining of the nucleocytoplasmic norm, and the organism divided into unequal parts in the plane which was median before the operation. Lewis (1928) believes that the constancy of cytonuclear ratio (17 : 1) which he finds in the epidermal cells of the cucumber, both before and immediately after cell division, indicates that the cytonuclear volume relationship can not be a stimulus to cell division. From measurements of nuclei and cells in the primary meristems of a number of the higher plants, Abele (1936) believes that cell division follows a disproportion in the

ratio of nuclear surface to cell surface in the primary meristems of the higher plants.

Various factors, aside from the cytonuclear ratio, have been looked upon as the inciting cause of cell division. Boveri (1904) believed that the size of the chromosomes is responsible for initiating division. The chromosomes divide when they reach a size double that which they had at the close of the preceding division. At the same time he showed that the rhythm of the division of the centrosomes may be independent of that of the chromosomes and that division of the cell depends upon the centrosomal rhythm rather than upon the chromosomal rhythm. Conklin (1912), in his work on cleavage stages in *Crepidula,* shows that the results of his measurements "do not indicate that the Kern-plasma-relation of Hertwig is either a constant or self-regulating ratio in the blastomeres of these eggs; on the other hand, it appears to be a result rather than a cause of the rate of cell division, and consequently it is a variable rather than a constant factor." He maintains that changes in this ratio are not causes of such cell activities as division but results of the metabolic processes by which such activities are brought about (a view shared by Woodruff, 1913), and that the factors which bring on cell division are to be found in some intrinsic condition in the nucleus or centrosome, rather than in a constant ratio of nuclear volume to cell volume. Gurwitsch (1909) maintains that the blastomeres are ready for division at all times, and that various causes, aside from nucleocytoplasmic volume relations, may be the immediate stimulus to cell division.

<center>POLYPLOIDY</center>

The cytonuclear ratio has frequently been studied in cases of polyploidy. In 1902 Gerassimow obtained races of *Spirogyra* with double the original nuclear volume and found that the size of a cell is a function of the mass of its nuclear substance. Boveri (1905) showed that the sizes of both nucleus and cell in polyploid echinoderm larvae are dependent upon the chromosome number. With an increase in the number of chromosomes, the nuclear volume grows faster than the corresponding cell volume, and the surface of the nucleus, rather than its volume, is proportional to the number of chromosomes; the size of the cell is proportional to nuclear surface and chromosome number. Erdmann (1908) con-

cluded from investigations of successive cleavage stages of sea urchins that chromosomal mass, rather than the constant chromosomal number is related to cell size. Gates (1909) also found the size of cells of giant tetraploid individuals of *Oenothera* to be dependent upon nuclear size. Similar results were obtained from a study of tomatoes and nightshades by Winkler (1916). Lindstrom and Humphrey (1933) found cell size and nuclear size to be related to chromosome number in tetraploid tomatoes.

Several studies have been made on the relation of chromosome number, nuclear size and cell size in polyploid mosses which have been produced by artificial apospory. The Marchals (1909) found the volumes of nuclei and cells of *Amblystegium, Physcomitrium* and *Funaria* to be directly proportional to the number of chromosomes. Cell and nuclear volumes of the tetraploids are approximately twice those of the diploids; those of the diploid, twice those of the haploid. Wettstein (1924) found an increase in cell size in polyploid mosses, but the increase was not directly proportional to chromosome number. Very little is said about nuclear volume in Wettstein's work. If one assumes proportionality between chromosome number and nuclear volume (Lindstrom and Humphrey, 1933; Osawa, 1913), then the volume of the nucleus evidently increases less rapidly than the volume of the cell.

The polyploid relationships discussed thus far are those which have been found in *induced* polyploids. In such forms there appears to be a close correlation between chromosome number, nuclear size and cell size. In *natural* polyploids, however, body size, organ size and cell size are, in general, not very different from their normal size in diploids. Gaiser (1927) found a general lack of uniform relation between number of chromosomes, cell size, organ size and plant stature in races of *Anthurium*.

CONCLUSIONS

Of the various conceptions in the literature as to size relationships of cell to nucleus, some were refuted quite early, and others have required certain modifications. There is no absolute value for the cytonuclear ratio applicable to all cells; none that applies to all cells of a species, nor even to those of a given tissue. There is a fluctuation of the ratio corresponding to the mitotic cycle; there are progressive changes of the ratio in development and differen-

tiation; there are differences of ratio between divergent cell types of the same age; and there are specific differences.

But for any homogeneous sample or comparable series, the ratio, though not static, is not random, but often follows a definite law. Thus in a series we may expect neither uniformity of cell and nuclear size, nor a constant ratio between them, but a uniformity in *relative rate of change*. For instance, in a series from meristematic cells to any one type of their mature derivatives, one may expect a much more rapid increase of the cell than of the nucleus, so that the absolute value of the C/N ratio increases with age. The *relative rate of change* of cell and nucleus, however, is often consistent, and in such cases a logarithmic plotting of the data gives a straight line. The slope of this line gives a figure characteristic of the relationship, whereas no single cytonuclear ratio can be characteristic. However, in a given developmental series, the absolute cytonuclear ratio may well be a characteristic of the stage as is the known age or absolute size of the cell.

Some confusion in the literature comes from differences in the terminology used. The K/P and C/N ratios, varying immensely, would seem, to the unwary, to give opposite trends for the same data, and further the gathering of some data in terms of volume, of others in terms of surface has made strict comparison more difficult. Partial reconciliation of aparently divergent views can sometimes be brought about by reducing the results to a common basis.

Even more serious differences concerning the significance of the cytonuclear ratio have come from too wide generalizations by different authors using different types of material. This was probably true of the classic controversy of Hertwig and Minot who gave opposite interpretations of the meaning of the ratio for senescence, basing their theories on Protozoa and vertebrates, respectively. The confusion in this field, however, has been extended by the too strict use of arbitrary definitions of senescence secondarily based on the C/N ratios. For instance, there is a well known trend in animals as in plants for increase of the C/N ratio from the embryonic tissues through the stages of differentiation and "senescence." But except for the definition of senescence derived from this trend, few people would otherwise consider the zygote (with high C/N) as senescent and in need of the "rejuvenescence" brought about by the decrease of the ratio during cleavage.

Very little can be said about the effect of temperature on the cytonuclear ratio. As has been pointed out already, innate differences in the material may account, in part, for the fact that some workers find a direct proportionality, and others, an indirect proportionality between the ratio and temperature, and some find no consistent temperature effect.

So also it appears to be impossible to draw any adequate conclusions as to the effect of the ratio on cell division. There is definitely a considerable nuclear increase (*i.e.*, fall of the cytonuclear ratio) immediately preceding division; but whether this change is a stimulus to the ensuing division (Hertwig, 1903), or is a mere accompaniment of the preliminary changes, is not known.

Other approaches than this one of size relationship are undoubtedly necessary for a complete understanding of all the physiological implications of the interdependence of nucleus and cell. The biochemical study of the ratio, between total nitrogen and purine nitrogen or other representatives of the nucleus has given comparable but more exact measures of these processes. It should in the future allow distinction between consistent shifts in the ratio of active masses (as in development) and accessory changes in size due to dilution of cytoplasm or nuclear material. In this way it may elucidate cases where it is now doubtful whether surface or volume changes are the more significant. Until such chemical measurements are more widely applied to plant cells or until we have a surer knowledge of the metabolic physiology involved, a knowledge of the simpler quantitative relations is a first step toward understanding. Size measurements offer a convenient and still very fruitful approach, though an indirect one, to the important biological problem of the functional relation between nucleus and cytoplasm.

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THE BOTANICAL REVIEW

NUCLEOLI AND RELATED NUCLEAR STRUCTURES

R. RUGGLES GATES

University of London, King's College and Marine Biological Laboratory, Woods Hole, Mass.

CONTENTS

INTRODUCTION—HISTORICAL REFERENCES

The nucleolus is an organ of the cell which is almost universally present and yet whose function and history have until recently remained obscure. The cytological work of the last few years has, however, thrown a great deal of light on the origin and history of the nucleolus in the cell. This work, which has been especially extensive and significant in plants, shows that the numbers, sizes and exact points of origin of the nucleoli on the chromosomes in the nuclei of any species can be used in a comparative way, together with other evidence, in tracing the phylogeny of the species within a genus and can even provide evidence concerning the origin of

certain genera and larger groups. Very recently, new methods of investigation have thrown considerable light on the biochemical composition of nucleoli which, combined with a knowledge of the history of the nucleoli in the mitotic cycle, brings much nearer a full understanding of the rôle of these bodies in the metabolism and reproduction of the cell.

The number of nucleoli arising in the nuclei in telophase can now be regarded as characteristic of the species. Before the following prophase they have usually merged into one. This body is a viscous semi-solid, denser than the karyolymph and frequently containing vacuoles and crystalloids. It has a different refractive index and in some cases can be thrown out of the nucleus by centrifuging.

While this account will be concerned mainly with the conditions in plant cells, reference will also be made to some of the more significant contributions on the nucleoli of animal cells, which are more highly specialized in certain tissues.

As stated by Henneguy[1] (1896, p. 6), Fontana (1781) figured the nucleus in the epithelial cells of the eel, and described it as a round or oval body with a spot in the middle. This spot we now call the nucleolus. Wagner (1835) discovered the nucleolus in the germinal vesicle of *Unio* and *Anodonta,* as pointed out by Montgomery (1898). He called it a Keimfleck or macula germinativa. Further references to the early history of the nucleolus are given by Carnoy (1884). In Vol. I of his "Repertorium für Anatomie und Physiologie" (1836), Valentin describes (p. 144) how, having macerated an eye in water for 16–24 hours, he examined the epithelial cell layer of the conjunctiva. He says: "Die Kerne dagegen zeigen sich durch die Einwirkung des Wassers mehr oder minder angeschwollen. Das Körperchen in der Mitte ist in der Regel nicht mehr sichtbar.[2] Dagegen haben viele Nuclei einen hellen Fleck im Centrum, während ihre Kügelchen mehr die Peripherie einnehmen." The word nucleolus is not used in this volume.

In Vol. IV (1839), which is entitled "Die Fortschritte der Physiologie im Jahre 1838," and is in the form of a review of all current work, Valentin says (p. 5): "Man erfuhr, dass zuerst der Nucleolus (das Kernkörperchen) und der Nucleus (der Kern) entstehe und dann um diese herum nicht sowohl einseitig, wie das

[1] I am indebted to Dr. E. G. Conklin for this reference.
[2] Nucleoli have recently been observed to be soluble in water.

Uhrglas auf der Uhr, sondern concentrisch die neue primäre Zellenwand mit ihrem durchsichtigen Inhalte sich herumlagert," from which it is clear that he accepted the current notion that the nucleolus produced the nucleus and it in turn produced the cell. He also says, however: "entsteht oft in der fertigen Mutterzelle (durch Theilung ihres Kernes?) eine Zahl neuer Kerne, von deren jeder sich mit einer neuen Zelle umgiebt, während in gleichem Maase als diese neuen Zellen wachsen, die Mutterzelle resorbiert wird und endlich ganz schwindet," which is the old conception of free cell formation. He continues: "An diese Beobachtungsreihe knüpft sich das Resultat der Bemühung eines anderen Forschers, dass die gebildete Zelle sich oft dadurch vermehre, dass sich in ihr neue primäre Doppelscheidewand bilde und dass dann so durch Theilung aus einer Zelle zwei entstehen." He thus accepts somewhat doubtfully this "third method" by which cells can multiply, namely, by division into two, without naming the author to whose observations he refers. Regarding cambium, he says (p. 57): "Ganz eigenthümlich ist das Cambium der Baüme, welches sich zuerst als strukturloser organischer Stoff ergiesst und aus dem unmittelbar das Prosenchyma ohne Vermittelung von Cytoblasten, ohne formation von Zellen in Zellen, entsteht." Without entering further into questions of the cell theory, we may quote again (p. 7): "So bildet also die Zelle mit Nucleus und Nucleolus gewissermassen die Grundgestalt der pflanzlichen und die thierischen Gewebe, also der Elementarorgane in der organischen Welt überhaupt." From which it appears that Valentin's views were the same as those of Schwann. Valentin used the terms "nucleolus" and "Kernkörperchen" and refers to its presence in many tissues. The latter term was also used by Schwann (1839).

Carnoy (1884, p. 174) says: "Après Fontana, ce fut Valentin qui décrivit et représenta le premier le nucléole, dans son Repertorium, t. i, 1836. Il dit du nucléole qu'il est un petit corps rond, *une espèce de second noyau* [his italics] à l'intérieur du premier: 'rundes Körperchen welches eine Art von zweiten *Nucleus* bilde.'" I have not been able to find this quotation in the original, but Valentin says (Vol. IV, p. 276), in comparing animal with plant tissues: "auch hier Zellen in Zellen um die zuerst abgelagerten Nuclei entstehen." On p. 277 he says, in describing the cell clusters in cartilage: "Sie enthalten einen oder mehrere kugelrunde

oder etwas ovale, wie es scheint, hohle Kerne mit zwei dunkelen Kernkörperchen. Im Innern liegen oft mehrere freie junge Zellen mit Nucleus und Nucleolus."

Henneguy (1896) states that Valentin described the nucleus in cells of the conjunctiva and pointed out a round corpuscle, the nucleole, in its interior, forming "a kind of second nucleus" within the nucleus. The term nucleolus was used in a paper by Bowman (1840) on the structure of muscle fibres, in which he refers to Valentin and figures a "loose cell" containing a nucleus, which in turn contained a nucleolus.

Schwann (1839) who, together with Schleiden, has long been erroneously credited with the origin of the cell theory, was so impressed by this simple arrangement of one globule within another that, as recently pointed out by Conklin (1941), he supposed that the inner granule (nucleolus) expanded into a nucleus, which in turn formed the cell. He held that this process of cell formation could also take place in a formless ground-substance, the cytoblastema. Schwann gives the credit to Schleiden (not Valentin nor Wagner) for having discovered in onion cells a small corpuscle (nucleolus) within the nucleus, but as the earliest appearance of Schleiden's "Beiträge zur Phytogenesis" was in Müller's Archiv, 1838, the credit for this discovery belongs to Wagner and Valentin. In Schleiden's observations he noted one to four nucleoli in a single nucleus. He described the formation of a new cell from the cytoblast (nucleus) by expansion from within; and although he also found a mode of formation of new cells by the division of existing cells through the formation of partition walls, he thought this might be an illusion! Thus, the fact that with the crude methods then available little or nothing was visible in many cells except the outlines of cell, nucleus and nucleolus, made possible a sort of emboîtement theory of the reproduction of cells—a theory which was as wide of the mark in cytology as was Bonnet's theory of development by unfolding in embryology. It is evident that the unfolding theory of structure and development—like rabbits out of a hat—exercised great influence on men's minds until the growth of chemistry and physiology laid the groundwork for views based on metabolism.

The nucleolus long remained a mysterious body. Zacharias (1885) remarked: "Die physiologisches Bedeutung des Nucleolus

ist noch völlig unbekannt." He cites the well known view of Strasburger that it probably held the reserve material of the nucleus, and of Pfitzner that it was an accumulation of nutritious material for the formation of chromatin. Strasburger afterwards conjectured that the nuceolus might be concerned in producing the spindle, a view for which there has never been any evidence. Zacharias concluded that it was not a reserve accumulation, and he agreed with Flemming that it is a cell organ of unknown function. Strasburger regarded its rôle in the cell as passive, pointing out its lack of organization. But Zacharias disagrees and argues that even the fusion of nuceloli and their fragmentation does not prove the inactive character of these bodies. It remained for greatly improved technique to show much more recently that the nucleoli do not merely float freely in the nuclear sap, but that they arise from and normally remain in contact with certain loci of the chromosomes.

The well known terms "plasmosome" and "karyosome" were introduced by Ogata (1883), the karyosome containing chromatin (it was called a "chromatin nucleolus" by Montgomery, 1898) while the plasmosome is paler, staining with basic dyes. It is the significant body on which the modern work has been done, and very little reference will be made to karyosomes.

Montgomery (1895) in a well known monograph, summarized all that was known of the nucleolus up to practically the end of the last century. Extensive observations had been made of the nucleoli in the nucleus of animal egg cells and of various glandular and other tissue cells, in some of which the nucleolus took on specialized functions. Many observations had been made, for instance, linking the nucleolus with yolk formation. Some were even led to question whether the nucleoli of egg cell nuclei were homologous with those of somatic cells. Montgomery answered this question in the affirmative, since both appear to be depositions of substances concerned in the nutrition of the cell. Montgomery, however, misinterpreted the relation of nucleoli in the egg cell to yolk formation. From the frequent occurrence of nuceloli on the periphery of the nucleus, just inside the nuclear membrane, he drew the conclusion that the nucleolar substance had an extra-nuclear origin in the cytoplasm. It is now well recognized that the movement of nucleolar material is in the opposite direction.

NUCLEOLAR EXTRUSION IN ANIMAL CELLS

It is desirable to cite here a few of the many papers which have finally proved that in the egg nuclei of many animals the nucleoli multiply and are finally extruded bodily through the nuclear membrane into the cytoplasm, where they take part in the formation of yolk substances. The earliest paper I have seen in which this conclusion is drawn, is that of Lubbock (1861). In the egg of *Geophilus* he describes and roughly figures the process which we would now understand as the formation of nuclear protrusions, each containing a nucleolus, and he connected this process with yolk formation. But he cites Leuckart, "Untersuchungen z. Natur. des Mens. und d. Thiere," 1858, as having described a similar but more regular "subdivision of the Purkinjean vesicle" (nucleus) in the egg of *Aphis*.

Balbiani (1883) describes and figures the formation of diverticula from the egg nucleus, each of which contains a body. He thought that these diverticula formed small nuclei while the nucleolus remained in a central position and took no part in the process. Scharff (1887) found in the younger fish ova a great number of nucleoli, mostly in contact with the nuclear membrane but a few in the center of the nucleus. He figures the stage with "peculiar protuberances" all over the surface of the egg nucleus, forming diverticula. Calderwood (1892) observed budding of the nucleoli into the cytoplasm in the eggs of the conger. He connected the process with the formation of oil globules. Eggert (1929) figures clearly the extrusion of nucleoli through bulges in the nuclear membrane of the egg of *Salarius*.

Ludford (1922) summarized the literature on the nucleolus in animal cells during the first two decades of this century and described the nucleolar behaviour in a mollusc. He refers to many papers on yolk formation and other extrusions from the nucleus. In the youngest oocytes of *Limnaea* no nucleolus could be seen. Later, the nucleolus grows in size and performs amoeboid movements, portions of it being extruded into the cytoplasm. The nucleolus then becomes clearly differentiated into oxyphil and basophil portions, the oxyphilic part continuing to extrude portions of itself into the cytoplasm, especially towards the end of oogenesis. The basophilic part finally breaks up into a granular substance distributed throughout the nucleus, these granules forming aggregates on

the reticulum. Ludford (1924), in a study of melanosis in the horse, described two forms of extrusion from the nucleolus: *a*) the nucleolus extrudes fragments directly into the cytoplasm; *b*) little buds are formed from the nucleolus, which are discharged through the nuclear membrane into the cytoplasm. Melanin granules were often found in vacuoles inside the nucleus.

Hogben (1920), in a study of oogenesis in Periplaneta, described the young oocyte as containing one nucleolus (plasmosome). At first, minute deeply staining particles are emitted from it, which migrate through the nuclear membrane to the periphery of the cytoplasm, where they disappear. Then arise bodies which are termed deutosomes, which pass through the nuclear membrane into the cytoplasm, the nucleolus becoming vacuolate. This latter process synchronizes with the beginning of yolk formation. Gatenby (1922), in describing the egg development of *Saccocirrus,* finds that the nucleolus forms small buds which are extruded into the perinuclear zone of the cytoplasm. Striking appearances are shown in later stages of yolk formation. That conditions of yolk formation may be different in members of the same group is shown by Nath (1925). In two species of scorpions with yolk which is insoluble in fat solvents, the nucleolus of the egg grows in size and emits into the cytoplasm deeply basophil, round bodies. In another genus whose eggs have no such yolk the nucleolus remains inactive.

W. H. Lewis (1922) first described the extrusion of the nucleolus into the cytoplasm in tissue culture, in fixed preparations of endothelium grown *in vitro*. He regarded the process as more or less pathological, but Ludford (1925*b*) observed stages in the extrusion of a portion of the nucleolus into the cytoplasm in living cells (fibroblasts) of the rat's kidney in tissue culture and regarded the process as normal. He also (Ludford, 1925*a*) observed the extrusion of portions of the nucleolus in the cells of the epididymus of the mouse. Hett (1937*b*), on the other hand, finds in the epithelial cells of the epididymus and vas deferens of man, that vacuoles develop in the nucleus and pour their contents, which may be homogeneous or granular, into the cytoplasm, the nucleolus being apparently not involved in the process.

Jorgensen (1913) made an elaborate study of yolk formation in the eggs of invertebrates, illustrated with many colored plates. He found that the eggs of many animal classes, especially Mollusca,

have two nucleolar substances, Patella having three. There is generally one nucleolus in young oocytes. They increase by (*a*) spontaneously appearing in larger number, (*b*) budding, (*c*) becoming amoeboid, or (*d*) growing into ribbon-like or chromosome-like strands. He concludes that the nucleoli of different animal eggs are wholly different in composition and hence not comparable with each other. Gardiner (1927) concluded, contrary to other work, that the nucleolus in the egg of *Limulus* arises from the confluence of substance which passes from the cytoplasm into the nucleus, but she found that the greater part of its substance afterwards passes back into the cytoplasm in yolk formation. Using the Bell and Doisy test for organic phosphorus, she found that the nucleolus is richer in phosphorus than other cell constituents. Nucleolar emissions were believed to transfer phosphorus from the nucleus to the cytoplasm in lecithin formation, the nucleus putting out pseudopodium-like protrusions. The Needhams (1930), in a study of phosphorus metabolism in invertebrate eggs found that the extent to which lipoid phosphorus is transformed into other forms of phosphorus in the embryo is proportional to the amount of lipoid present at fertilization, some eggs having large stores and others very small amounts.

Brief reference may be made to two other papers showing the part played by the nucleolus in yolk formation. Subramaniam (1935) finds in oocytes of *Clibanarius* that the nucleolus buds repeatedly, the buds passing through the nuclear membrane into the cytoplasm where they grow and bud further and finally disappear in the peripheral cytoplasm. After budding, the nucleolus grows again, probably by material derived from the cytoplasm. It is suggested that the mitochondria also grow by utilizing extruded nucleolar material and that there is condensation by the Golgi apparatus of nucleolar material dissolved in the cytoplasm. The part played by mitochondria and Golgi bodies in yolk formation can not of course be considered here. Subramaniam and Aiyar (1935) find a somewhat different condition in the eggs of a teleost. In the youngest oocyte the nucleus contains a number of scattered nucleoli. Later, these migrate to the nuclear membrane and undergo a change, staining like fat globules with Sudan III. In living eggs the nucleoli could be seen attached to the nuclear membrane and forming protrusions like pseudopodia, thus confirming Lubbock.

A recent study of the nucleoli in frog's eggs (Gersch, 1940) yields further interesting information and conclusions. In the ripe eggs of *Rana temporaria* and *R. esculenta* the volume of the nucleus is found to be about one fifty-thousandth that of the oocyte. The youngest oocytes have a single nucleolus which divides by constriction into very many larger and smaller nucleoli scattered throughout the nucleus. In the ripe egg of *R. temporaria* only a few small nucleoli remain in the centre of the nucleus, while in *R. esculenta* they are scattered. In both species the number of nucleoli greatly increases during yolk formation.

It is found that in four developmental stages of the egg the nucleoli differ in solubility, the change beginning with yolk formation. In young oocytes the nucleoli are resistant to distilled water, but in later stages they dissolve; similarly with concentrated NaCl. The conclusion is reached that the nucleoli contain higher proteins, probably phosphonuclear proteins. Both the nuclear sap and plasma of oocytes contain glutathione or a similar protein having the sulfhydryl group. These increase during yolk formation and from this and other evidence glutathione may perhaps be present in the nucleolus. It contains phosphorus but rarely or never thymonucleic acid, and the tests for potassium and iron are negative. The isoelectric point of the nucleoli was found to be, in younger oocytes pH 4.4, later 3.6. Gersch concludes that the nucleoli carry on specific metabolic changes for each type of cell and furnish materials for various cell processes. There is no nuclear extrusion during yolk formation, but some nucleoli dissolve and may pass in this form through the nuclear membrane.

Oka (1940) finds that in the young eggs of a Holothurian one large nucleolus is present, sometimes two. This is extruded through a pocket in the nuclear membrane, a young nucleolus being formed to take its place. Such extrusion is said to take place several times successively. Does the young nucleolus grow from a chromosome locus? The nucleus is about 35.5μ and the nucleolus 7.5μ in diameter.

It thus appears to be established that in many animal eggs, even when relatively little yolk is present, the nucleolus is highly specialized in its metabolism and actively gives off bodies which pass into the cytoplasm and there take part in the formation of yolk, including fat and other substances. In various other specialized tissues

and gland cells similar processes take place. As examples of the latter kind may be cited Schreiner (1915), who found in the fat cells of *Myxine* that the lipoid granules stored in the cytoplasm are derived from the nucleolus. Hett (1937a) similarly found in the granulosa lutein cells of the hedgehog that the nucleolus takes up a peripheral position against the nuclear membrane, which becomes thin and permeable at a narrow point through which the nucleolar material passes and spreads out into the cytoplasm.

In secretory plants cells various studies have been made which indicate special nuclear activities, but they have not been carried out with sufficiently critical methods to make clear the course of events. Kruck (1931), for example, makes numerous observations on the nucleolus in the secretory cells of *Utricularia* bladders. She finds that after the plant has fed, the nuclei of the bladder cells move to the inner wall of the cell, the nucleoli becoming paler staining. The Randnucleoli which she describes may be prochromosomes.

FEULGEN STAINING

The introduction of the Feulgen reaction for chromatin has led to entirely new and fundamental developments in the study of the nucleus. This staining method made it possible not only to identify the chromatin in every stage of nuclear development, but to show that the nucleolus (plasmosome) does not contain chromatin. By this means many of the older theories, according to which the nucleolus manufactured chromatin, have been eliminated. These various views, discussed in Wilson (1925) and Sharp (1934), are now mainly of historical interest. The chromatoid body described by Wilson (1913) in the spermatogenesis of *Pentatoma* as simulating an X-chromosome could be confused with a chromosome only in the absence of a stain for chromatin such as we now have. It is a cytoplasmic body which appears outside the nucleus during the growth period of spermatogenesis. It does not divide and finally passes into one of the four spermatids, later wandering into the sperm-tail where it is cast off. In the first meiotic metaphase it could easily be mistaken for a chromosome in preparations stained with iron-haematoxylin or similar stains. Schrader (1940) has recently shown, using the Feulgen reaction, that this body does not contain chromatin.

Feulgen (1924) states that he had found many years ago that

when the purine base is split off from thymonucleic acid by mild acid hydrolysis the free reducing group gives a violet color with fuchsin sulphurous acid (aldehyde reaction). Sugars do not give this reaction in acid solution because they are in the tautomeric cyclic form. Pentose-containing nucleic acids (yeast nucleic acid, guanylic acid, inosinic acid) do not give it because their carbohydrate is a sugar (d-ribose). This reaction—the staining with fuchsin sulphurous acid (Schiff's reagent) after hydrolysis—he called the nucleal reaction. With over-hydrolysis it is a frequent experience in my laboratory that plant cell walls and other parts of the cell may show the violet coloration. There remains some doubt, however, whether this reaction can be regarded as a test for the presence of the –CHO group. It has been pointed out (Semmens, 1940) that the heterocyclic compounds pyridine and piperidine give the Feulgen reaction, but this is attributed to their basicity.

With the introduction of the Feulgen reaction for chromatin the modern remarkable developments in knowledge of the nucleoli began. For example, Bauer (1933) tested the Feulgen reaction on the oocytes of many insects. In nearly all species the nucleolus stained strongly with Heidenhain but was uncolored by Feulgen, showing that there was no transfer of chromatin from nucleolus to chromosomes. The nucleolus in the egg of *Stegomyia,* however, and the periphery of that in *Anopheles,* were Feulgen-positive.

Yuasa (1937) found that leaf cells of Pteridophytes are Feulgen negative when fixed in chromic acid of more than a certain strength. This may be because the thymonucleic acid is destroyed by the oxidizing power of the chromic acid.

It soon became evident that a counterstain for the nucleoli was necessary for the further study of these bodies, and in my laboratory such a stain was worked out (Semmens and Badhuri, 1939; Badhuri, 1938, 1941a). It consists simply in the ordinary Feulgen stain followed by mordanting with sodium carbonate. Then a short stain in light green or fast green and the green can easily be washed out of the cytoplasm. The nucleoli remain specifically stained green. The sheath of the chromosomes can also take the green stain in metaphase, and in early telophase the scattered fragments of this sheath take the same color for a short period until they are absorbed into the growing nucleoli. The stain is permanent, can be applied to either paraffin sections or smears, and preparations have shown no deterioration after more than a year.

Heitz (1936) first introduced the smear technique for root-tips. In the hydrolysis with HCl which precedes the Feulgen stain the middle lamella of the cell walls is broken down so that, in smearing, the cells are easily spread into groups and rows which are one cell thick. Hillary (1939, 1940) has also recently used the Feulgen stain together with fast green and finds, contrary to our earlier experience, that acetic acid in the fixative is not deleterious to the subsequent staining. In using the stain for any new genus it is necessary to find the combination of acetic acid and formalin which gives the best effect and also to determine the optimum time of hydrolysis. Permanent smear preparations can now be completed in a matter of hours, not days, and will last without fading much longer than gentian-violet preparations. Delay (1940) has also obtained red chromosomes and green nucleoli in Leguminosae by using Feulgen and vert lumière, mordanting with sodium bisulphite and HCl.

Toluidine blue and thionin have recently been used (Francini, 1939) for a contrasting stain of nucleolus and chromatin. Root tips of *Paphiopedilum spicerianum* were stained in toluidin blue. Anthers of *Nigella sativa* in thionin show the nucleolus red, the nuclear reticulum blue-violet and the cytoplasm pink-violet after a few days. Anthers of *Paeonia moutan* show the chromosomes violet, nucleolus red, cytoplasm pale violet, cell walls red. Other work with the nucleous leads the author to the conclusion that it is of dual composition: *a*) a protein portion which is not stained with Heidenhain; *b*) a stained portion which is a sulphuric ester of a polysaccharide and resembles pectin in staining, *e.g.,* red with ruthenium red. This part is believed to pass into the karylymph in prophase. It reappears in telophase as small granules which later aggregate in the nucleolus. This aspect will be discussed more fully below.

Zirkle (1928, 1930) showed by using different fixations that the nucleoli do not contain chromatin. He revived the term plastin for the nucleolar composition and concluded that in telophase the nucleolar material which had been incorporated into the chromosomes collects into globules which fuse to form the nucleolus. The plastin which has thus been in intimate contact with the genes passes out into the cytoplasm in the next mitosis and so serves as a vehicle for the transmission of influences from the genes to the

organism. The plastin, also being electro-positive, changes the charge on the chromosomes from negative to positive. Kuwada and Sugimoto (1928) also found that in resting nuclei the chromatin is electronegative and the nucleolus electropositive, in agreement with cataphoresis experiments, the chromatin being blue and the nuceoli red in neutral violet extra.

NUCLEOLI IN LOWER ORGANISMS

Before taking up the modern work which has proved the relation between nucleoli and satellite chromosomes in higher plants and animals, it is desirable to refer to certain conditions in the lower organisms. Using the Feulgen stain, it has been shown (Spearing, 1937) that while the lower Cyanophyceae (*Oscillatoria*) contain chromatin and some species contain in their cells rod-like chromatin bodies which divide transversely, there is no nucleolus. In the higher Cyanophyceae (*Stigonema*), on the other hand, although the division of the chromatin network is amitotic and there is no nuclear membrane, a definite globular body like a true nucleolus is present.

There were formerly many accounts of mitosis in algae, fungi and invertebrates in which it was claimed that the chromosomes in prophase arose within the nucleolus. This seemed so contradictory to the history of the chromosomes in higher organisms that these accounts were not generally accepted. The difficulty has recently been resolved by investigations of the nuclear conditions in various algae. Geitler (1935) has shown in *Spirogyra* that the chromosomes arise in prophase in the karyolymph in the usual way, but that they then migrate into the nucleolus. In one species, after the split chromosomes have entered the paler staining nucleolus, they form there a metaphase plate and separate, the nucleolus meantime undergoing a mass division in anaphase, or, in some species, disappearing altogether in metaphase. In a second paper, essentially the same results are shown by means of the Feulgen stain. Svedelius (1937) has observed essentially the same conditions in the red alga *Lomentaria rosea*. The division in the tetrasporangium is not a reduction division. The large nucleolus is free from chromatin and about 20 chromosomes arise in the karyolymph in prophase. They then migrate into the nucleolus and there divide, separating into two anaphase groups.

The process of mitosis in these algae may therefore be spoken of as *intranucleolar*. By telophase in *L. rosea* the nucleolus has disintegrated and the nuclear membrane presumably breaks down. In *L. clavellosa,* on the contrary, meiosis takes place in the tetraspore mother cell and is of the ordinary type, the chromosomes not migrating into the nuceolus. Why this migration takes place is not at all clear unless the chromosomes add nucleolar substance to their content. Westbrook (1935) concluded from studies of several red algae that the chromosomes are not formed in the nucleolus, but she did not recognize the evidence that they subsequently migrate into this body. Intranuclear mitosis has frequently been described in various Protista, but intranucleolar mitosis is a newly recognized phenomenon.

In early studies of animal spermatogenesis, the fact that nucleoli or parts of them stained like chromatin with haematoxylin and other dyes often led to misleading conclusions. It is now clear, however, that in various animals the chromosomes move into the plasmosome in essentially the way just described in algae. Blackman (1903) described in the spermatogonia and spermatocytes of Myriapods all the chromatin aggregating into a mass named the karyosphere, from which the chromosomes afterwards arise. In a later paper (1905), the plasmosome is found to change into the karyosphere, growing in size partly by the "deposition of chromatin on its periphery." Later, the chromatin is described as leaving the karyosphere in masses which break up to form the chromosomes.

McGill (1906) emphasizes the double character of the nucleoli in dragon-fly oogenesis. She found the nucleoli to consist of oxyphil (paranuclein) and basiphil (nuclein) portions which may unite to form one body, usually with the basiphil portion surrounding a central oxyphil portion in many animals. The basiphil substance was generally supposed to be chromatin, which shows how misleading a stain may be. In resting nuclei all the chromatin was believed to pass into the nucleolus and to pass out again into the chromosomes in mitosis. Flemming's triple stain and Obst's borax-carmine and methyl green method gave sharply contrasted colors to these materials. The double nucleoli were believed to arise by condensation of basiphil chromatin around the oxyphil nucleolus. In an earlier study of echinoderm eggs, Guenther (1903) found

double nucleoli, each with a central oxyphil substance surrounded by a basiphil layer which he supposed to be chromatin.   In fresh starfish eggs the nucleolus can in fact generally be seen with a high-power lens to contain a central droplet surrounded by an outer portion with a different refractive index, but neither is chromatin (Gates, 1942).

Browne (1913) later showed in three species of *Notonecta* that in the growth period of the spermatocytes the chromosomes are aggregated to form a karyosphere consisting of "chromatic bodies embedded in plasmosome materials."   Although she was uncertain how this body arose, it seems clear now that the chromosomes arise in the usual way in prophase, passing into the nucleolus and later passing out again, the process being similar in many respects to that in the algae already described.

Saville (1939) has recently recognized a new type of nuclear behavior in the rust fungi.   Using the Feulgen reaction, the rusts are shown to have an "unexpanded" nucleus wherever passage through a narrow pore is necessary.   This changes to the expanded type by the formation of an ectosphere from the surrounding cytoplasm.   The chromatin then passes entirely into the ectosphere. The endosphere is not a nucleolus, as hitherto supposed, but it contains a small body which is probably the true nucleolus.

In Protozoa, Reichenow (1928) showed with the Feulgen reaction that both the macronucleus and micronucleus of such genera as *Colpoda, Chilodon* and *Urostyla* contain thymonucleic acid.   M. S. Lucas (1930) found that in *Paramecium* both macronucleus and micronucleus give a brilliant purple with Feulgen, while in *Balantidium coli* the former was purple, the latter pale violet or negative. In *Chaos* and *Opalina* granules or masses in the nucleus stained purple.   From further observations, Lucas and Evans (1935) concluded that saprozoic Protozoa contain little or no thymonucleic acid.

In the trophozoite nucleus of a myxosporidian (Kudo, 1922) the large karyosome divides before the nucleus divides.   The two halves then differentiate, the karyosome in the vegetative nucleus remaining large while that in the generative nucleus gets smaller. In some Protozoa (Wenrich, 1941) the nuclei may stain with Feulgen at one stage of the life cycle and not at another.   This appears to be because the Feulgen-positive chromatin is highly dispersed in

the chromosome material, where it can be seen as tiny, faintly coloured granules.   In another protozoan, *Ceratomyxa* (Noble, 1941), the karyosome stains with Feulgen, like the peripheral chromatin of the nucleus.   It is formed, at least in part, from the chromosomes, but divides to form two centrioles which soon disintegrate.   It may contain both nucleolar and chromatin material.

The situation as regards the nucleoli in certain Protozoa has recently been made clear by the work of Chen (1936).   He finds that in the ciliate *Zelleriella intermedia,* the 12 pairs of chromosomes differ, like the chromosomes of many higher organisms, in size, in the position of the spindle fibre attachment and in the presence of nucleoli on certain pairs.   Two races were found, one having two pairs and the other three pairs of nucleoli, although the chromosome number was the same in both.   This is a situation which has been paralleled in higher plants such as rice.   The nucleolar pairs differ in size, a very small pair being associated with chromosome No. 6 near the middle of the short arm, resembling the nucleoli ("chromosome vesicles") of Orthoptera.   Chromosome No. 1 has a very large nucleolus along the mid-region of the long arm, while chromosome No. 4 has a large nucleolus in the middle region of the long arm, extending along much of its length. In another species, three races were found having, respectively, 6, 8 and 14 nucleoli.

These nucleoli of the ciliates were formerly called macrochromosomes, but they do not stain with Feulgen.   They differ from the nucleoli of higher organisms in that they are generally elongated, surrounding the chromosome for a considerable distance.   The chromosome is not contracted to a thread at this point, but can be seen as one or two chromatids through the surrounding pale nucleolar material.   The nucleoli also divide in metaphase and are generally carried to the poles with their respective chromosomes in anaphase.   Thus they differ in several minor respects from the nucleoli of Metazoa and Metaphyta and may perhaps be regarded as somewhat less specialized.   Ahrens (1939) has described in the oogenesis of a Copepod with $n = 11$ chromosomes, three nucleoli attached terminally to three bivalent chromosomes.   In the young oocyte, when yolk granules are forming in the cytoplasm, the nucleus shows only a spindle-shaped nucleolus with a fine thread at either end attaching it to the nuclear membrane.   In

later prophase, three "nucleolomeres" (satellites?) can be seen attaching the three chromosomes to the nucleolus. The presence of three such chromosomes requires elucidation in relation to chromosome number.

MODIFIED MITOSIS

In chick embryos and other rapidly growing embryonic tissues where mitoses are seldom seen, it has frequently been suspected that some other form of nuclear division must take place. Stough (1931) seems to have shown that this is the case in the mesenchyme, striated muscle and other syncytial tissues of the chick embryo. At first sight, in these tissues there appears to be a "chromatin nucleolus" which divides by constriction into two. Further study leads to the conception of a "modified mitosis" in which the "nucleolus" is recognized as a mulberry-like cluster of very small chromosomes. These presumably divide and are seen to form two masses connected by delicate fibres in anaphase, the whole giving the appearance of a dumbbell. The process is intranuclear and a membrane laid down on the spindle finally divides the nucleus into two. The amount of chromatin is only a fraction of that in adjacent cells undergoing mitosis. No nucleolus is mentioned and apparently none is present. Perhaps this phenomenon can be interpreted in the following way. The chromosomes are so small because they lack a sheath and are reduced to the essential genic materials of the threads in the resting nucleus. There being no sheath or matrix to the chromosomes, no nucleoli are formed in telophase and the whole nucleolar cycle is omitted.[3]

Similar conditions have been seen in embryos of fishes, amphibians, birds and mammals, so it may be characteristic of vertebrates. In a later paper, Stough (1935) has found from a large number of counts that only 1.48% of the cells in chick embryos are in ordinary mitosis at any one time, which seems quite inadequate to account for the rate of growth. He also finds transition nuclei, in which ordinary telophase passes directly into the mulberry masses of modified mitosis. It is suggested that in such nuclei and tissues the chromatin remaining may have only a restricted function for the needs of the specified tissues. There is perhaps a condition comparable with the chromatin elimination in *Ascaris*. This could be

[3] It will be shown later that the nucleolar material is derived from the chromosome sheath.

determined only by comparing more fully these minute bodies in the "mulberry" with the chromosomes of an ordinary metaphase. On the whole, it seems more likely that all the chromosomes are represented, but reduced to their lowest terms, *i.e.,* stripped of everything except their genic materials.

In the nervous tissues there appears to be quite generally one nucleolus in each nucleus. This is to be expected generally in resting nuclei which are not undergoing mitosis. Davenport and Ransom (1931), in their extensive counts of nerve cells and fibres, say that "cells were enumerated by counting nucleoli." Lucas (1940) states, however, that in normal cells of the lamina epithelialis of the fox brain there is a chromatin network but no nucleolus. This needs confirmation with the Feulgen-light green stain after mordanting with sodium carbonate.

"NUCLEOLI" IN VIRUS-INFECTED ANIMAL AND PLANT CELLS

A number of animal viruses produce intranuclear inclusions, some of which resemble nucleoli but are presumably of different character. Findlay (1932) described a certain strain of mice in which every individual had inclusions in some of its liver cell nuclei. Only 1% of the nuclei were affected. They contained one or more round bodies 8–90 µ in diameter. The small ones stained faintly with Feulgen, the larger ones a rose pink. This was interpreted as probably nucleolar hypertrophy, but since they contained chromatin these bodies were probably not true nucleoli. Although affected mice showed no other symptoms, this was evidently a mild pathogenic virus, because mice could be infected by an emulsion of liver cells which had been passed through a Berkefeld filter. Cowdry and Kitchen (1930) showed that in monkeys the nucleoli of the liver cells are amphoteric, *i.e.,* they have a core of oxyphilic (basic) material covered by a layer of acidophilic material which, until the Feulgen reaction, was interpreted as chromatin. In yellow fever of monkeys, intranuclear inclusions are formed in the hepatic cells. The nucleolus enlarges, the "chromatin" layer becomes attenuated and inclusion masses develop in the karyoplasm. In human yellow fever the condition of the liver cells is described as "hypertrophy of the nucleoli." Probably in all such cases, while the true nucleoli may be modified by apposition of other substances or otherwise, the inclusions are of different character and should not be called nucleoli.

Lucas (1940), using Feulgen and other stains, in a study of fox encephalitis, another virus disease, finds characteristic necrotic changes in the nuclei of certain brain tissues.   There is an increase in the Feulgen-positive chromatin granules, which enlarge while the nucleus shrinks to form a pycnotic nucleus, the nuclear sap also in the meantime staining magenta with Feulgen.   In other cells a large, central, Feulgen-positive (basichromatin) inclusion body forms.   The oxyphil material ("oxychromatin") separates from the basichromatin and finally the whole inclusion body becomes Feulgen positive.   By centrifuging, the "oxychromatin" is found to have a lower specific gravity than the basichromatin.   In infected cells the basichromatin is heaviest, then the inclusion body, its two parts (basi- and oxy-) tending to separate.

From these and many other results with normal and pathological tissues it can perhaps be concluded that the nucleolus in animal cells normally contains acidophil and basiphil portions or constituents (although these terms have not always been consistently used), but clearly neither of these is chromatin as was formerly supposed. Recent observations of my own on living eggs of *Asterias* and *Mactra* (Gates; 1942) show that the nucleolus (under an immersion lens) consists of two parts or substances, one generally enclosed within the other.   In Mactra there are sometimes two nucleoli, the larger one containing an internal body.   These evidently correspond to the oxyphil and basiphil portions described by histologists.   When fresh water is run under the coverslip the outer portion of the nucleolus quickly dissolves while the inner body is much more persistent and may survive long after the other part has disappeared.   These two parts sometimes come to lie side by side or can be seen to separate before the larger one disappears.   Gersch (1940) found that the nucleoli of frog's eggs are completely soluble in distilled water in the later stages of their development, before laying.

Severe etch is the only plant virus disease in which nuclear inclusions are known.   In infected tobacco plants (Sheffield, 1941) several nucleoli are present and plate-like crystals appear in the nuclear sap.   These bodies are not formed at the expense of the nucleoli and they show some differences in staining capacity.   With the Feulgen-light green stain the nucleoli and the inclusion crystals both stain green, but with pyronin B the nucleoli are red and the inclusions colorless.

CHEMICAL COMPOSITION OF NUCLEOLI

Only recently have attempts been made to determine directly the chemical nature of different nuclear constituents. Shinke and Shigenaga (1933), using the various histochemical tests for thymonucleic acid, proteins and lipoids, concluded that the nucleolus contains lipoids, but this observation and others were too indefinite to be altogether satisfactory or conclusive. They concluded that the nuclear reticulum and chromosomes contain nucleo-proteins united with thymonucleic acid, together with some lipoids, while the chromosome matrix contains lipoids combined to form lipoproteins. That the nucleolus and the chromosome matrix contain similar substances, there is other evidence which will be considered later. In metabolism, lipoids apparently arise from nucleoproteins, not from nucleic acids. The presence of what are regarded as protein crystalloids in plant nucleoli has long been known.

Yasui (1938) studied acetocarmine smears of the pollen mother cells and tapetum of *Papaver, Hosta, Tradescantia* and *Magnolia*. He observed lipoid droplets arising from the tapetum and the pollen mother cell nuclei. They assumed myelin forms in three days and were believed to be lecithin. As the chromosomes degenerate in old preparations, oily droplets arise from them, and in resting nuclei they arise from the nucleoli. In the light of observations to be detailed later, such droplets would probably be formed from the chromosome sheath, which appears to be related in composition to that of the nucleoli. They suggest that the slow separation of the lecithin-like substance from the resting nucleus is because it does not exist free but in combination. These droplets were found to arise only from the chromosomes and nucleoli, not from the karyolymph.

Mensinkai (1939b) made tests of the nucleoli in root-tips of *Allium*. He found that they were coloured orange-red with Sudan III and black with 1% osmic acid. He concluded that the nucleoli contain much lipoid material and that they serve as a basic source of energy for the cell and the organism. Claude (1940), by using the high speed centrifuge on normal and tumor cells, has separated small granules of uniform chemical constitution with a diameter approximately that of mitochondria. The latter are known to consist largely of phospholipids and proteins. These particles are found to contain a phospholipid-ribonucleoprotein complex. Now

it is known that mitochondria aggregate around the nucleus in the prophase of mitosis, and this is the position in which Caspersson and Schultz (1940) find a high concentration of ribose nucleic acid, which may therefore be contained in the mitochondria.   The phospholipids isolated by Claude contain aldehyde groups and therefore give the Feulgen reaction.   They are probably the acetalphosphatids of Feulgen and Bersin (1939).   If lipoids exist in nucleoli they are quite possibly also phospholipids, in which case the nucleoli of fresh cells should give the Feulgen reaction without hydrolysis.[4]

A more exact method of studying the composition of nucleoli and other cell organs is that of Caspersson (1939) who used the ultra-violet absorption spectrum of various substances for this purpose.   By such measurements in living cells the amount of nucleic acid was found to be constant from mid-leptotene through meiosis. Nucleic acid is found to increase in the chromosomes during somatic prophase, confirming conclusions drawn from general microscopical studies of mitosis.   It is concluded that desoxyribosenucleic acid is necessary for division of animal cells.   In later papers, Caspersson and Schultz (1940) applied the ultra-violet method to show that ribonucleic acid are present both in the nucleolus and (1939) in the cytoplasm of rapidly growing tissues, such as onion root-tips. Dividing eggs of sea urchin, chick and fish are shown also to contain pentose nucleic acid.   Caspersson (1936) had shown that the absorption spectrum of nucleic acid gives a striking curve with a maximum at 2600 Å in the middle of the ultra-violet, due to the conjugated double bonds of the constituent pyrimidine rings.[4a]

The nucleic acids fall into two groups according to the carbohydrate they contain: a) desoxyribose of which thymonucleic acid is the type (in animals); the latter was formerly supposed to be responsible for the staining reactions of chromatin; b) ribose nucleic acid, to which yeast nucleic acid belongs (in plants).   The latter are composed of phosphoric acid linked with the purine and pyrimidine bases by a carbohydrate.   On hydrolysis they yield phosphoric acid; pentose; the purine bases, guanine and adenine;

[4] Later experiments (Gates, 1942) have shown that substances of this character are not found in the nucleolus but in the cytoplasm of animal eggs.

[4a] The specificity of this method may, however, be less than at first appeared. The absorption spectrum of ribonuclease and other protein enzymes has a maximum at 2800 Å, minimum at 2520 Å (Uber and Ells, 1941).   Also crystalline trypsin has a max. at 2800 Å and min. at ca. 2537 Å (Uber and McLaren, 1941).

and the pyrimidine bases, cytosine and uracil in plants or thymine (methyl uracil) in animals.

Caspersson and Schultz (1940) show a high concentration of ribose nucleic acids around the nuclear membrane, but these do not stain with Feulgen. The ultra-violet spectrum absorption curve for the nucleolus shows a maximum at 2600 Å and a hump near 2800 Å. The latter probably represents proteins containing tyrosine and tryptophane. The nuclear sap and diffuse chromatin show the presence of both nucleic acid and protein, the absorption curve for the latter being largely determined by the amino acids. They, however, have found no good evidence for the presence of lipoids in the nucleolus. The old theory of the migration of nucleic acid from the cytoplasm to the nucleus is evidently contrary to fact. Each has its own nucleic acid cycle. As the nucleolus generally dissolves in the cytoplasm during mitosis, some ribose nucleic acid from the nucleus would reach the cytoplasm in this way. On grounds to be explained later, while it is recognized that the chromonema contains thymonucleic acid, it is quite possible that the chromosome sheath or matrix contains ribose nucleic acid which in telophase becomes aggregated into the nucleoli.

It has also been suggested that nucleoli contain glutathione, on the rather indefinite evidence of the presence of a sulfhydryl (–SH) grouping. This radicle appears to be associated with increase in cell number, *i.e.*, mitosis, rather than cell size (Hammett, 1938). The presence of glutathione as well as vitamins A and C appears to have been demonstrated in mitochondria, however, which are purely cytoplasmic bodies.

NUCLEOLAR SIZE

Various measurements have been made of the comparative size of nucleoli, and certain recent studies of variation in nucleolar size are of considerable significance in connection with the functions of this body. Conklin (1912), in an exhaustive study of cell and nuclear size in *Crepidula*, found that the size of the nucleoli (plasmosomes) depends on the size of the nucleus and the length of the resting period since the last mitosis. When nuclear division is delayed by treatment, the nucleoli become much larger than normal. Bretschneider and Hirsch (1937) have recently shown, on the other hand, that in eggs of *Lima* (Lamellibranch) the nucleus and

nucleolus at first grow coincidently; then the latter stops while the former continues its growth.

Heitz (1925) shows that in the regeneration of mosses and liverworts, while the chloroplasts get smaller the nuclei and nucleoli get larger.   This is believed to be due to storage of sugar.   In *Lophocolea* the nuclear volume of the regenerative cells in relation to that of normal cells was 5:1.   The corresponding relation in the nucleoli was 12:1, *i.e.,* the nucleoli enlarge proportionally much more than the nuclei.   Fortak (1931) made some observations on the cells of the germinating embryo of *Peperomia blanda.*   He found that some cells, which have larger nucleoli, later become oil cells.   The stoma mother cells also have larger nucleoli, while in the surface cell layer of the radicle the nucleoli are very small.   In the aleurone layer bordering the perisperm, which produces enzymes on germination, the large nucleolus breaks into small granules which go into solution.   These observations are in accord with Ziegenspeck's hypothesis that the nucleolus produces enzymes and contains proenzymes.

Fischer (1934) shows that the nuclei in the wound zone of a leaf of *Peperomia blanda* enlarge.   He found, like Conklin in animal cells, that if cell division is inhibited the nucleolus gets larger.   In *Peperomia* the growth of nucleoli is less than that of nuclei, while in *Bryophyllum* it is greater.   The nucleolar size is found to be much influenced by nutrition.   If leaves of *Bryophyllum* are kept in darkness the nucleoli are smaller after three days and they may even disappear.   There is also a great diurnal variation in nucleolar size, which might be regarded as a pulsating activity of the nucleus. Between afternoon and morning the difference in mean volume of the nucleoli may be 26%–50%.   If pieces of leaves are grown in sugar solution the nucleoli are larger than in pieces kept in darkness, and in general the nucleoli are larger the more carbohydrate is present in the leaf.   These results would all indicate that the nucleolus is an important storage organ, presumably of sugars, in the leaf cells.

Deloffre (1939) finds that the traumatism of sectioning causes notable growth of the nucleus and nucleolus in adjacent cells of the embryonic axis of the lupin.   The mean diameter of nuclei and nucleoli at time of sectioning was 15.3 µ and 3.9 µ, respectively. They grew steadily over a period of 40 hours to mean sizes of 18.2 µ

and 6.8 μ, respectively, and then began a period of decrease.   These observations may be subject to a different interpretation.

Sayles (1927) found that in the regeneration of *Lumbriculus* after wounding, the nuclei and nucleoli are much larger in endodermal, mesodermal and ectodermal cells. He regards large nucleoli as an indication of high metabolic activity of the cells.

Meyer (1918) quotes results of Zacharias with the nucleoli of *Galtonia* leaves. He compared the mean nucleolar volume in the leaves of a normal plant, a plant kept in darkness 36 days, a plant after two months in darkness, and found them to be, respectively, 1.0 : 0.38 : 0.18. Lukjanow similarly found that in starved animals the nucleolus decreased in size faster than the cells. Meyer himself measured the nucleolar volume in *Galtonia* from a vegetative leaf-base in July, a bulb scale in November, and a leaf-base from a resting bulb in December. He found them to be in the ratio of 15 : 30 : 34 and concluded that the nucleoli contain reserve materials. He quotes other results and suggests that as nucleoli are present in the ganglion cells of animals the nucleolar protein is useful not only in growing but also in differentiated cells. He concludes that as the nucleoli disappear in the cytoplasm during mitosis perhaps nucleolar proteins can exist only in the nucleus. He observed that young nuclei in telophase have several nucleoli which later fuse.

Hartmann (1919) observed that animal embryos have very large nucleoli. He found in *Cladocera* that the nucleolar : nuclear ratio decreases with increase in cell size and in age of the organism. He gives many tables of measurements and finds that decrease of cell metabolism is accompanied by increase in size of the nucleoli. At the time of birth a specially strong reduction in nucleolar volume takes place, which is believed to indicate a decrease in the intensity of nucleolar metabolism.

It has been found (Loretti and Perroncito, 1938) that if the parotid glands of a certain mouse are made hyperactive by pilocarpin, gigantism of the nuclei and nucleoli results. This is believed to be due to decrease in the viscosity of the cytoplasm and the resulting changes in surface relationships.

As regards nucleolar number in somatic cells, de Mol (1928) made it clear that diploid hyacinths have a maximum of two, triploids three and tetraploids four. He found that these are all nor-

mally of the same size in hyacinths; but he made the false assumption that they increase from one by fragmentation, whereas it is now clear that each type of cell begins in telophase with the maximum number of nucleoli, this number being subsequently reduced to one by fusion.   De Mol (1937) has since shown that whereas *Narcissus pseudonarcissus* and *N. poeticus* ($2n = 14$) each have two nucleoli, the amphidiploid ($2n = 28$) produced from a cross between them is a cell giant with larger flowers and four nucleoli. As regards nucleolar size, de Mol (1928) found that in a given variety in cells with one (compound) nucleolus it is larger than the sum of the nucleolar volumes when there are two or more.   Bhatia (1938) has confirmed this relationship by measurement of nucleoli in the leptotene pollen mother cells of tetraploid and hexaploid wheat having, respectively, a maximum of four and six nucleoli, as shown in the following table of measurements.

TABLE I

	4 n		6 n	
	Cells measured	Total volume of nucleoli	Cells measured	Total volume of nucleoli
1 nucleolus	12	704.0	9	1021.7
2 nucleoli	13	555.1	11	885.5
3 nucleoli	13	486.9	7	709.8
4 nucleoli	6	473.8	1	737.0

It will be seen that the total nucleolar volume is greatest, in both the 4 n and 6 n wheat, when the nucleoli have fused into one, and diminishes progressively where there has been less fusion.   This Bhatia attributes to greater growth of the nucleolus in the former case, but it may also be due to the inclusion of other substance between the fusing nucleoli.   Similarly, Parthasarathy (1938) found in the pollen mother cells of rice at diakinesis that the volume of the single fusion nucleolus was greater than the sum of the volumes when there were two.   He attributed this to the fact that the single nucleolus has less surface through which loss of material to the nucleus can take place.   It will be seen from Table I that the volume of nucleolar material is consistently greater in the hexaploid than in the tetraploid.   This would be expected if each genome generally contains one nucleolus-producing chromosome.

Ramanujam (1937) found, on the other hand, in an autotriploid rice with three satellites and three nucleoli that in the root-tip cells (60 measurements) there was no significant increase in nucleolar volume of the triploid compared with the diploid. Dermen (1933) found similarly in *Petunia* polyploids no correlation between size of nucleolus and number of chromosomes. Parthasarathy (1938) compared the mean nucleolar volume (100 measurements) in root-tip nuclei of diploid and haploid rice plants. The ratio was 489.9 : 212.2 : or over 2 : 1. Pathak (1940*a*) compared the nucleolar volume in the diploid cells of a root with that in a tetraploid sector of the same root in *Crocus sativus* var. *Elwesii* and found the volumes approximately 2 : 1. It appears that generally a change in the ploidy of the plant causes a corresponding increase or decrease of nucleolar material in the cell, but in certain cases some readjustment takes place.

VanCamp (1924) has made certain measurements which are of interest from another point of view. There is a sense in which the meristem of a stem-tip constitutes a Keimbahn (Gates, 1910) and it has long been recognized that embryonic and meristematic cells have conspicuously larger nucleoli. Measurements of seedlings by vanCamp show that in the meristem the ratio of nuclear volume to nucleolar volume is 16 : 1, whereas in the rootcap cells it is 30 : 1 and in fully differentiated cells it is *ca.* 40 : 1. He concluded that the nucleolar material was not a waste product.

Further interesting size relationships of the nuclei and nucleoli in varieties of rice are brought out in a paper by Selim (1930). He compared varieties from five different countries and the results are embodied in Table II. The var. Kochivittu generally had two large nucleoli in its pollen mother cells, Nabatat practically always had one, while the other varieties fell in a series between. When two nucleoli were present they were attached. From Table II it will be seen that while the nuclear volume in the pollen mother cells is virtually the same in all, the nucleolar volume shows a decreasing series, both relative and absolute. Nandi (1937) compared the nuclei and nucleoli in pollen mother cells of six varieties of rice from India, China and Japan. He found them the same size in all, but in each variety the nuclear diameter and nucleolar volume were greater when there were two nucleoli than when there was one in the cell. He concluded that the size of the nucleolus depends on

the size of the nucleus. Selim supposed that the second nucleolus in the pollen mother cells was produced by budding from the first and that budding took place only when the nucleolus reached a certain size. Nandi (1937) showed that in zygotene the threads of what we would now call the nucleolar chromosomes lie between the two nucleoli in pairing. Parthasarathy (1938) and Pathak (1940c) showed that of 20 rice varieties examined, 12 have four nucleoli in somatic cells and eight have two. The variety Kochivittu probably belongs to the former class and Nabatat to the latter.

TABLE II

Variety	Usual nucleolar condition in meiotic prophase	Nucleolar Vol. / Nuclear Vol. in P. M. C.	Diameter of nucleus in meiotic prophase	Diameter of nucleolus before synizesis	Nucleolar Vol. / Nuclear Vol. in root-tips
Kochivittu, India	2 large nucleoli	5.29%	24.8	6.7	9.75%
Egyptian var.	1 large, 1 small nucleoli	3.37%	24.5	6.4	.....
Temas, Java	1 large, 1 small nucleoli	2.92%	24.4	6.0	.....
Japanese 6	1 nucleolus	2.62%	24.5	5.7	.....
Nabatat, Persia	1 nucleolus	2.55%	24.4	5.6	6.5%

Selim found that the "secondary nucleolus," which he assumed was budded off from the primary, was paler staining and disappeared earlier, therefore contained different materials. In the light of present knowledge, these primary and secondary nucleoli would each represent a pair. The slight differences in their staining and behaviour indicates that their content is somewhat different. This may account for the stability of the condition with two pairs of nucleoli in so many species. Many cases of supposed nucleolar budding in pollen mother cells are due to pairing or fusion, but there are others where a true budding appears to take place.

Woods (1937) compared the nuclear and nucleolar volumes in three tulip varieties. The results are shown in Table III and the percentage volume of $\dfrac{\text{nucleolus}}{\text{nucleus}}$ is given in an additional line.

TABLE III

		Adonis (2 n)	Gipsy (2 n)	Inglescombe yellow (3 n)
Root-tips	Mean nuclear vol.	646.7	996.1	916.3
	Mean nucleolar vol.	18.4	37.8	32.1
	$\dfrac{\text{Nucleolar vol.}}{\text{Nuclear vol.}}$	2.8%	3.8%	3.5%
Meiotic prophase	Mean nuclear vol.	725.8	710.3	1446.8
	Mean nucleolar vol.	10.0	8.8	11.2
	$\dfrac{\text{Nucleolar vol.}}{\text{Nuclear vol.}}$	1.37%	1.23%	0.77%
Pollen tetrads	Mean nuclear vol.	153.7	262.8	195.9
	Mean nucleolar vol.	1.3	2.1	2.6
	$\dfrac{\text{Nucleolar vol.}}{\text{Nuclear vol.}}$	0.84%	0.79%	1.33%

From this table it will be seen that the nucleoli are relatively larger in root tips than in the pollen mother cells.

NUCLEOLI AND SATELLITES

This has proved to be the most significant and extensive line of advance in connection with nucleolar study. Reviews of papers bearing on the discovery of the relation between the nucleolus and the chromosomes have been given elsewhere (Gates, 1937, 1938), so that only the essential points need be briefly summarized here. S. Navashin (1912) discovered the satellites or trabants in *Galtonia* as minute globular bodies attached to the nucleolus in prophase. He thought that they afterwards became attached to the end of certain chromosomes and were thus pulled away from the nucleolus. We know now that the satellite is a part of certain chromosomes, to which it is permanently attached by a tenuous thread, and that each nucleolus takes its origin in telophase from the end of the chromosome, at the point where the satellite thread emerges. McClintock (1934) has called this point the "nucleolar organizing body." It has been assumed by Heitz (1931), who discovered that each nucleolus arises on a satellite chromosome in telophase, that the thread or sat-stalk[5] also plays a part in producing the nucleolus,

[5] The term SAT-thread was first used by Heitz (1931) as meaning *sine acido thymonucleinico,* but various investigators soon showed that the thread frequently stains with Feulgen. As Sat happens to be short for satellite, it

and others have supposed that the satellite as well plays a part in its formation.   The terminal locus of the chromosome body appears, however, to be the only region which is essential in producing a nucleolus, although certain facts suggest that these other structures may in some cases play a part.   Navashin (1927) found that in somatic cells of *Galtonia* the satellites can be seen in the resting nuclei as pycnotic bodies attached to the nucleolus.   Berger (1941) has pointed out that in *Spinacia oleracea* ($n=6$), in roottips of which $2n$, $4n$ and $8n$ cells are frequently found, the heteropycnotic sats. in resting nuclei can be used as a method of determining the ploidy of the cells.   In a study of the very small chromosomes of *Salix*, Wilkinson (1941) finds that *S. alba* and *S. fragilis* are both allotetraploids having $2n=76$ chromosomes.   Both these species have two pairs of sat-chromosomes, while *S. alba* var. *caerulea* has only one pair.   He figures resting nuclei of *S. fragilis* with two pairs of pycnotic sats. attached to the nucleolus and of *S. caerulea* with two sats. similarly attached.

Another body of similar character was discovered in the nucleolus of the pollen mother cells of *Lathyrus* (Latter, 1926), and subsequently in pollen mother cells of *Lathraea, Oenothera, Malva, Oryza* and other plants.   This was called by Latter the "nucleolar body."   It is a part of the nucleolus, not of the chromosome, being formed at the point of attachment of one or more (satellite) chromosome pairs.   With Heidenhain's haematoxylin it stains more deeply than the rest of the nucleolus.   It is not chromatin, however, as was formerly supposed, since it does not stain with Feulgen, although it takes the gentian violet stain when the rest of the nucleolus is destained (Pathak, 1940*b*, Sikka, 1940*a*).   Remembering that the large nucleolus in pollen mother cells is a compound body formed by the fusion of at least two, and in hexaploid species (*e.g., Triticum vulgare;* Bhatia, 1938) of as many as six nucleoli, two or more nucleolar bodies may be present, but they have usually joined into one as the chromosome threads pair in zygotene.   They are generally on the surface of the fusion nucleolus.   In the pro-

seems desirable to continue its use in that sense.   Resende (1939) believes that the sat-thread, which he finds to be frequently Feulgen-negative, is so fine that material long fixed in a chromic acid fixative might give the Feulgen reaction even though it does not contain chromatin.   He uses the term sat-zone instead of sat-thread, because in *Encephalartos* and *Trillium ovatum* it is broad and pale, containing several pairs of chromomeres.   Fernandes (1936) calls this the "nucleologenic region."
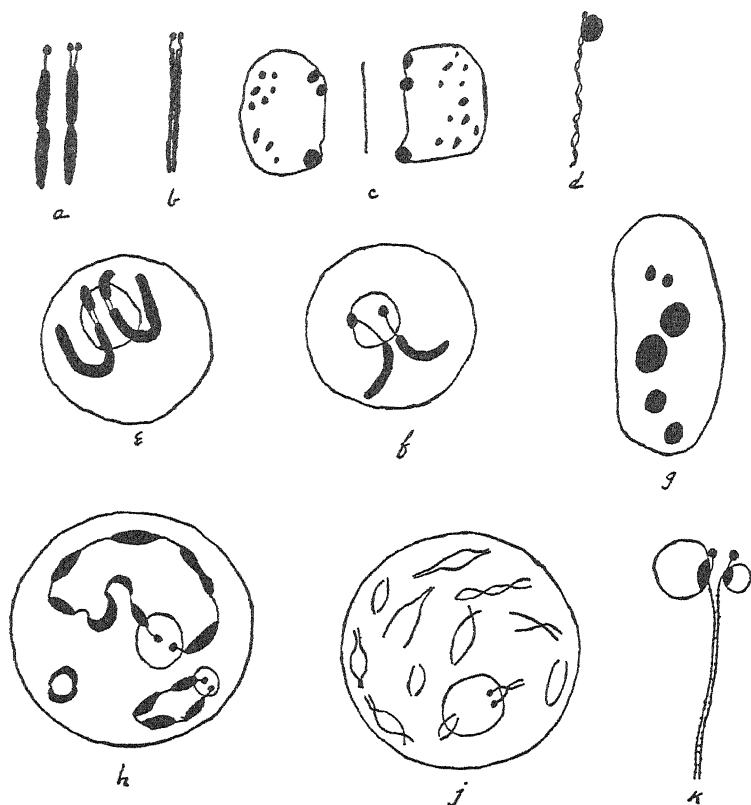
Fig. 1

FIG. 1. Diagrams showing the nucleolar cycle in mitosis and the relation of sat-chromosomes to nucleoli in meiotic prophase.

    a, Somatic metaphase or anaphase chromosome.

    b, Origin of nucleolus from a sat-chromosome in early telophase.

    c, Evanescent stage of telophase, showing three nucleoli and many droplets from chromosome sheath.

    d, A sat-chromosome in later telophase.

    e, Prophase in rye, showing two chromosomes with secondary constrictions attached to nucleolus.

    f, Prophase showing two sat-chromosomes attached to nucleolus.

    g, Telophase in hexaploid *Triticum spelta,* showing three pairs of nucleoli of different sizes.

    h, Diakinesis in an Oenothera hybrid, showing a ring of six, ring of four, and one pair. A pair of sat-chromosomes in each ring with satellites oriented towards each other.

    j, Diakinesis in rice, showing two bivalents attached to nucleolus.

    k, Zygotene in rice, showing relations of nucleolar body, nucleolar organizer, satellites and threads.

phase thread stages of meiosis six leptotene threads may be attached at different points to the surface of the fusion nucleolus in hexaploid wheats and four in tetraploid wheats (Bhatia, 1938).

Thus the nucleolar body in pollen mother cells is a part of the nucleolus, while the nucleolar organizing body is a part of the chromosome. Yet they must be in contact at the point where the nucleolus-producing chromosomes are attached to the nucleolus they produce. They have not been seen in the nucleoli of somatic prophases, possibly because they would be very small. Further researches are necessary to elucidate this relationship. It should be further pointed out that while the nucleolar body in pollen mother cells is relatively large and easily demonstrated with proper staining, the nucleolar organizer of telophase sat-chromosomes is an area which is undifferentiated, except that the sat-thread takes its origin at that point.

Since this was written, a critical study of a paper by Carothers (1913) shows that the nucleolar body in meiotic plant cells has also been seen in animal cells during spermatogenesis. She described as many as three "chromatin vesicles," afterwards called "chromomere vesicles," in the meiotic nuclei of *Brachystola* and *Arphia*. These are really the nucleoli or plasmosomes. Carothers describes a "double vesicle" attached to the X-chromosome, and two single vesicles evidently attached to other chromosomes. These vesicles were colorless "except for a dense peripheral granule through which passes the spireme thread." This exactly corresponds with the description of Latter (1926) in the pollen mother cells of *Lathyrus,* except that in animal nuclei the structures are much smaller. Carothers describes and figures (see Figs. 7–16) a "minute, deeply staining granule at one point of the periphery" of the vesicle or nucleolus, which is always in connection with the chromatin, *i.e.,* at the point of attachment of the chromosome to the nucleolus. She points out that this body attached to the nucleolus has been figured without comment in the pachytene stage of human spermatogenesis by deWiniwarter (1912, Fig. 25).

These bodies, originally called "chromatin vesicles," were afterwards (Carothers, 1931) called "chromomere vesicles" when it was found that they were Feulgen-negative. They were shown to be associated in meiotic prophase with definite chromomeres of certain chromosomes, but little or no attention has been given to their numbers, which have proved so significant in plant nuclei. Recently,

further studies have been made of them. Corey (1940) found these nucleoli as a constant feature in the male germ cells of over 60 species of Orthoptera. In each species a pair of homologous chromosomes each bears "at or near the distal end" a vesicle associated with a particular chromomere or aggregate of chromomeres. The question may be asked, Is there here also a tenuous thread and a minute satellite, as in plant cells? These "vesicles" were also seen by Corey in somatic resting and dividing nuclei and in cleavage mitoses. They were associated with the chromonema only through a pycnotic granule or dense chromomere which is always at the periphery of the vesicle. This is evidently the nucleolar body of plant germ cell nuclei as already explained. The "vesicle" is found to be Feulgen-positive at certain stages. Two nucleoli are attached to a pair of homologous chromosomes in meiosis, and they are usually of unequal size, indicating a difference in the members of the pair. This is a pair of autosomes, but in the grasshoppers they are frequently heteropycnotic, like the X-chromosome with which they are associated. In Diptera, on the other hand, the sex chromosomes appear generally to produce the nucleoli as well.

Long (1940) has shown that in another orthopteran, *Scyllina cyanipes,* a pair of nucleoli is attached subterminally to an enlarged chromomeric thickening at the distal ends of a pair of homologous chromosomes. They become large and vacuolated, are seen at all stages of meiosis and at one stage show with Feulgen a peripheral ring of chromatin. When the nucleoli in animal species are counted, they will probably aid, as in plants, in the interpretation of .chromosome numbers. Ribbands (1941) has recently figured a dark area, evidently the nucleolar body, in the nucleolus during spermatogenesis in *Habropogon,* stained with gentian violet. He makes the useful suggestion that there is usually repulsion between euchromatin and nucleolar material. As heterochromatin is not repelled, the small sex chromosomes in the spermatogenesis of *Habropogon* are believed to be embedded in the nucleolar material although they have specific nucleolar attachments. But this explanation can hardly be general, because in many liverworts the sex chromosomes are heteropycnotic and yet show a sat. with a thread at the point of attachment to the nucleolus.

Significant results have been obtained by the study of the nucleoli in somatic and meiotic cells of *Oenothera.* In root-tips of many species four nucleoli are present in telophase. They generally

differ in size (Bhaduri, 1940), homozygous forms such as *O. Hookeri* and *O. blandina* having one large and one small pair while heterozygous species with a high degree of chromosome catenation, such as *O. Lamarckiana* and *O. Hazelae,* have one large, one small and two intermediate nucleoli. How these are "paired" is at present unknown. Prochromosomes are present, four of them generally attached to the nucleolus in somatic prophase. In a single form, *O. angustissima* var. *quebecensis,* five nucleoli were constantly present, with the five corresponding chromosomes attached to the nucleolus in prophase. How this condition has arisen is at present unknown. Another peculiarity of *Oenothera* is that the condition of the nucleolar chromosomes is more or less intermediate between that of a true globular satellite and a more elongated terminal portion beyond the secondary constriction. The distinction between a satellite and a secondary constriction therefore vanishes in this genus.

In the pollen mother cells of many *Oenothera* species and hybrids equally significant conditions have been found, especially where chromosome catenation is present. Two separate nucleoli are frequently present at diakinesis, each representing a pair of nucleoli with sat-chromosomes attached to them. The nucleolar bodies at the points of attachment can sometimes be clearly seen (Bhaduri, 1940). Now, it is known that in catenation the chromosomes in diakinesis are variously arranged end-to-end in fixed rings, and the evidence indicates that consecutive chromosomes in a ring are homologous, at least as regards their pairing ends. We may then expect to find in diakinesis two pairs of sat-chromosomes, each of which produces a (fused) nucleolus, and the two satellites of each pair should be oriented towards each other. When they are in a ring this ring should be completed and held together by the nucleolus. When the latter vanishes the ring becomes a chain, which arranges itself in zigzag manner in metaphase I.

All these conditions have been shown to exist, in a series of recent papers on *Oenothera* (Bhaduri, 1940; Jacob, 1940*b*; Pathak, 1940*b*; Sikka, 1940*a*). There are frequently two nucleoli of different size, each representing a fused pair.[6] In favorable cases it

[6] All workers with *Oenothera* have sometimes found, in addition to the regular nucleoli in the pollen mother cell nuclei, a variable number of small nucleolus-like bodies, the nature and origin of which are unknown, but they probably represent remnants of nucleolar material which have not been swept into the nucleoli.

can be shown that, *e.g.*, in a form with a ring of eight and a ring of four, two sat-chromosomes in the ring of eight with their satellites facing each other are attached to one nucleolus while in the ring of four two more sat-chromosomes are attached to the other fusion nucleolus (Jacob, 1940*a*). Often, however, the sat-chromosomes become detached from the nucleolus in fixation, but with gentian violet staining the nucleolar body can still be seen. By a study of the chromosome rings in a series of hybrids it thus becomes possible to identify the sat-chromosomes of different species by their place in the rings. A ring of 14 of course includes both pairs of sat-chromosomes. Further study of the satellites in various species and their hybrids will show in how far the sat-chromosomes are homologous from species to species.

It is significant that the related genus *Gaura* with $2 n = 14$ chromosomes also has four nucleoli, and *G. Lindheimeri* shows in diakinesis a ring of six chromosomes and four free pairs (Bhaduri, 1941*b*). Catenation has then probably developed independently as a parallel condition in both *Oenothera* and *Gaura*. The condition of four nucleoli in these two genera is probably older and perhaps indicates that seven is derived from an earlier basic chromosome number. Emerson (1929) found that in haploid *Oenothera franciscana* one bivalent was almost constantly present in the pollen mother cells, while a single chain of four was found. Catcheside (1932) found in haploid *O. blandina* the frequent presence of a bivalent and the rare occurrence of a trivalent or quadrivalent. These conditions might be accounted for by reduplication of segments, but this leaves the quadrinucleolar condition of the genus unexplained. Several other genera are now known to be in the same condition (see Table IV), which must therefore be regarded as highly stable. It seems likely to have arisen through some phylogenetic change in chromosome number, perhaps involving allotetraploidy, since the two pairs of nucleoli are unlike. In rice, where there are also four nucleoli, the whole nuclear history of the Oryzeae, including a change in basic number from 5 to 12, has been made clear by the work of Nandi (1936), Ramanujam (1938) and others. In *Nicotiana sylvestris* ($2n = 24$) the evidence may be interpreted to indicate three pairs of nucleolar chromosomes, the largest pair of nucleoli being produced by the pair of $SM_s{}^2$ chromosomes with secondary constrictions. The second pair of nucleolar

chromosomes have a terminal sat. and also a pycnotic area in the proximal arm.   The smallest pair of nucleoli are produced at a small terminal pycnotic area, there being no sat.   The "pycnotic knobs" require further study.

Some of the cases of apparent diploids with four nucleoli are brought together in Table IV, with some of their related species.

TABLE IV

	$2n$	Nucleoli	Author
Oenothera, all species examined but one ...	14	4 (2 large, 2 small)	Bhaduri, 1940
O. angustissima var. *quebecensis*	14	5	Bhaduri, 1940
Gaura Lindheimeri ...	14	4 (2 large, 2 small)	Bhaduri, 1941*b*
Gasteria obscura	14	4 (2 large, 2 small)	Sâto, 1936
G. maculata	28	8	Sâto, 1936
Aloinae 99 spp. ...		4	Resende, 1936
Aloinae 3 spp. ...		2	Resende, 1936
Aloe 7 spp. ...	14	4	Sâto, 1937
Aloe 3 spp. ...	14	2	Sâto, 1937
Aloe 4 spp. ...	14	3	Sâto, 1937
Aloe 1 sp. ...	14	5	Sâto, 1937
Gasteria 14 spp. ...	14	4	Sâto, 1937
Gasteria 3 spp. ...	14	3	Sâto, 1937
Gasteria 3 spp. ...	28	8 (only 6 seen in 2 spp.)	Sâto, 1937
Haworthia 13 spp.	14	4	Sâto, 1937
Haworthia 3 spp. ...	14	3	Sâto, 1937
Haworthia 2 spp. ...	42	(only 8 or 9 seen)	Sâto, 1937
Allium 2 spp. ...	16	4	Mensinkai, 1939*a*
Clitoria ternata	16	4	Jacob, 1940*a*
Crocus aerius var. Gray Lady	8	4	Pathak, 1940*a*
Hordeum vulgare	14	4	Heitz, 1931
Triticum monococcum	14	4	Pathak, 1940*c*
Galanthus nivalis	24	4	Sâto, 1938
Leucojum autumnale ..	14	4	Sâto, 1938

The significance of these and other results will be discussed later.

The precise origin of the nucleoli in telophase has been described with essentially the same details in plant and animal cells.   Dearing (1934) described it in *Amblystoma* and it was described by Gates and Pathak (1938) in *Crocus* (see also Pathak, 1940*a*).   In this genus the satellites are frequently on the long arm of a chromosome and these chromosomes project like fingers from the telophase nuclei.   The chromosome is composed of two strands, each with a sat. and its stalk.   With the Feulgen and light green stain a tiny green granule in contrast to the red chromosome can be seen at the

base of each stalk or thread. The two granules are close together. They represent the beginnings of the nucleolus and as they grow they shortly merge into one. This body by continued enlargement produces the full-sized nucleolus.

Instead of a pair of sat-chromosomes, a diploid plant occasionally has a pair of chromosomes with secondary constrictions. This is true, for instance, in rye (Pathak, 1939) which has $2n = 14$ chromosomes. This appears to mean simply that the nucleolar organizer with its accompanying constriction is situated farther back from the end of the chromosome. There are occasional cases where a reversal of an end segment of a sat-chromosome has taken place, so that the satellite and its thread are intercalary. This, however, is rare and it is probable that ordinary cases of secondary constriction have not arisen in this way. Rarely a species may have two pairs of chromosomes with secondary constrictions producing four nucleoli, but no satellites, as in *Allium sativum,* $2n = 16$ (Mensinkai, 1939a). Not infrequently an apparently diploid species may have one pair of sat-chromosomes and another pair with secondary constrictions. This condition exists, for example, in *Aegilops speltoides,* $2n = 14$ (Pathak, 1939); *Allium Cepa,* $2n = 16$ (Mensinkai, 1939a) and *Calceolaria integrifolia,* $2n = 18$ (Srinath, 1939) and suggests that these species may have arisen as allotetraploids, although another explanation is possible. Such species have four nucleoli in their telophase nuclei and these are frequently in two pairs of different sizes, indicating some difference in the activity of the nucleolar organizers. As already mentioned, the evidence for the origin of the Oryzeae through allotetraploidy is clear. The presence of two pairs of nucleoli in *Oryza* $(2n = 24)$ is supported by secondary pairing of the chromosome bivalents in meiosis (3 groups of two and 2 groups of three, or $3(2) + 2(3)$) which indicates that five is the basic number. This situation is further confirmed by the fact that in certain genera related to *Oryza* the chromosome numbers are high multiples of five, showing that five was the original basic number.

The metaphase condition of the chromosomes as regards satellites or secondary constrictions can thus be compared with the number of nucleoli in telophase and must also be confirmed by examination of the somatic prophase. Before this time in the mitotic cycle the nucleoli are generally all fused into one. The chromo-

somes which produced the original nucleoli will all naturally be found attached to this fusion nucleolus at the sat-thread or the secondary constriction as the case may be. With the contrast stain of Feulgen and light green or fast green these details can be precisely made out. It is no longer sufficient to publish a few drawings of the metaphase group with an occasional satellite as an indication of the chromosome condition in any species. This needs to be checked by comparison with other stages of mitosis, as already indicated. In many plant species, rice and *Trillium* for instance, the satellites of metaphase chromosomes are so small that they are easily overlooked even with the best technique. This method of checking observations is therefore essential. Comparison with the meiotic choromosomes, especially as regards secondary pairing, is also very desirable. In the result, by comparison of the conditions in related species considerable light is thrown on the nuclear phylogeny, which is an essential part of the species phylogeny. A new and important field of comparative cytology is thus initiated.

Now that these relationships are well established, it may be pointed out that the essentials as regards nucleolar numbers were already grasped by Yeates (1925) in a careful early paper on the nucleolus of *Tmesipteris tannensis*. He found that the maximum number of nucleoli was six in the sporophyte and three in the gametophyte. The nucleoli were found to arise *de novo* in telophase by the fusion of droplets, the nucleoli of sister cells often corresponding in position, number and size. The $2n$ chromosome number was ± 200. Yeates also pointed out the frequent association of the sex chromosomes with plasmosomes, but did not connect the nucleoli with sat-chromosomes. In this connection it should be remarked that Gates (1912) found in somatic cells of *Oenothera lata* "usually only three or four" nucleoli and figured a resting nucleus with two pairs.

THE NUCLEOLAR CYCLE IN MITOSIS

In his classical monograph on fertilization and cleavage in *Crepidula*, Conklin (1902) showed that each pronucleus has one nucleolus. In the telophase of the first cleavage the maternal and paternal chromosomes, which are in separate groups on the spindle, form separate daughter nuclei in contact, each having a nucleolus. Although nucleolar numbers were supposed until recently to have

no significance, Conklin, with prescience and logic remarks: "This persistence of a definite number of nucleoli in each telophase is a somewhat surprising fact and may possibly indicate that there is a persistence of some structure which may act as a centre for the formation of the nucleolus in each cell generation." We now know that this centre is a particular locus on a sat-chromosome, from which a fresh nucleolus arises in each mitotic cycle.

From the work of Mead (1898) and Lillie (1912) on Annelid eggs, however, it appears that in cleavage mitoses each chromosome forms a vesicle in telophase containing one or two nucleoli. There are probably no nucleolar organizers, or possibly each chromosome possesses one. The subject requires investigation.

From the evidence already assembled, the nucleolar cycle in mitosis is clear except at one point. The nucleoli arise in telophase at a particular locus of chromosomes having a satellite or a secondary constriction, the primary constriction being the point of spindle fibre attachment. Each grows to a predetermined size and when any two nucleoli touch during this growth or through movements of the chromosomes within the nucleus, they merge, like two droplets, into one. This process generally continues until before the following prophase a single large fusion nucleolus is present, to which the chromosomes which produced nucleoli will all be found attached at the loci of origin of the original nucleoli. When the nuclear membrane breaks down in late prophase the nucleolus generally becomes detached from the chromosomes and passes into the cytoplasm, where it disappears. The missing link in the nucleolar cycle is the source of the material from which new nucleoli arise in telophase. Considerable evidence now makes it clear that this material is derived from the matrix of the chromosomes.

In several different genera, such as *Narcissus* (Sikka, 1940b), *Crocus* (Pathak, 1940a) and *Cassia* (Jacob, 1940b), there is an evanescent stage of the somatic telophase in which numerous small angular or globular bodies are scattered throughout the daughter nuclei. They stain green, like the nucleoli, in Feulgen light green, but they are not the true nucleoli. They appear to be formed from the sheath or matrix of the chromosomes, which also frequently appears as a green line around the red chromosomes in metaphase. In early telophase this sheath breaks up into a large number of small masses which are used up and disappear as the true nucleoli

grow from their various loci. Sometimes a few of them remain as tiny "nucleoli," even into the resting stage. VanCamp (1924) figures in *Clivia* with Ehrlich-Biondi stain this stage in telophase in which scattered granules are present, but he describes them as simply flowing together to form the nucleolus. Whether the process is one merely of physical aggregation or, more probably, of chemical change, is uncertain. Others have figured this brief stage but have given it other interpretations. For instance, Resende (1937) figures three telophase nuclei in *Trillium erectum* with numerous "nucleoli" and regards this as an exception to the rule of a small fixed number of nucleoli. It probably represents this transient stage, or possibly the result of genomic derangement or the loss of a nucleolar organizer (see below). Such a stage of telophase is probably quite general in plant cells. The fact that it has been seen from time to time has prevented investigators from recognizing the fixed number of true nucleoli.

The nucleolar material is then derived from the matrix of the chromosomes and this completes the cycle of relationship between chromosomes and nucleoli. During prophase the nucleoli often diminish notably in size and some of their material is probably contributed to the formation of the chromosome sheath. McClintock (1934) has given a detailed account of how, in the pollen mother cells of maize, the pale staining sheath of the chromosomes can be seen to form irregular masses in telophase, which finally become organized to form the nucleolus. The evidence thus appears conclusive. In the absence of the organizer in X-rayed pollen mother cells (McClintock, 1934) all the material remains in the scattered condition. Further, a genomic deficiency, even when the organizer is present, may result in the nucleolar material not being collected at the nucleolar body. It is well known that a micronucleus containing a single non-sat-chromosome can still produce a nucleolus, which is presumably the unorganized material from the chromosome sheath.

To account for the growth of the nucleoli at the nucleolar organizers one may assume that each acts as a sink or sump at which the material aggregates. It is sufficiently liquid so that the nucleolus appears to be generally more or less globular at all stages of its growth. To account for the attraction of particles from the matrix fragments to the nucleolus one may assume that the or-

ganizer bears an electric charge which is stronger than that of surrounding regions (Mensinkai, 1939*b*). The ribo-nucleic acid content of the nucleolus may be derived from the chromosome sheath, as may also the lipoids if present. The sugars, which are believed to cause its diurnal variations in size in plant cells, must diffuse into the nucleus from the cytoplasm.

Among earlier investigators, several reached conclusions which are partly in agreement with those stated above. Fikry (1930) criticized the old view that the nucleolus elaborates chromatin and passes it on the "continuous spireme" in meiotic prophase. He pointed out the difficulties with any such transportation hypothesis and showed that the spireme in *Rumex* was not continuous. But he failed to find the nucleolar body and denied that any chromosome thread was attached to the nucleolus. Marshak (1931), by using various fixatives, established an intimate relation and possible identity between nucleolar material and the matrix substance of the chromosomes. Dermen (1933) concluded that the nucleolus is a by-product of the chromosome matrix, forming a chemical combination with some substance in the nuclear sap.

THE SATELLITE THREAD

The satellite thread or stalk may be merely the double chromonema of a chromosome stripped not only of its sheath but also of the outer wrappings of the chromonema itself, down to the condition of the threads in a resting nucleus. It is sometimes so tenuous as to be below the limits of visibility, but it has been definitely observed to be a spiral in certain cases and has also been considered (Mensinkai, 1939*b*) as a spiral of a lower order than the chromonemata in the body of the chromosome. Since metaphase and anaphase sat-chromosomes show the thread it has been assumed to be a permanent part of the chromosome. It appears that as the nucleolus grows in telophase the thread is stretched or despiralized, and sometimes the whole diameter of the nucleolus may intervene between the satellite and the body of the chromosome. If no nucleolus were formed at one locus, through the active competition of other nucleolar organizers, then the thread might be entirely retracted so that the satellite is attached directly to the chromosome. This may be what happens in cases of amphiplasty where, in hybrids of *Crepis* and others (M. Navashin, 1934) a satellite present

in one of the parent chromosomes disappears in the hybrid. Constant differences in the length of the thread also occur. Such cases require fuller investigation of the nucleolar conditions.

On the other hand, in genera such as *Allium* and *Narcissus* the length of the sat-thread is very variable in the cells of one plant. Mensinkai (1939*b*) accounts for this by the hypothesis that the satellite unrolls and so lengthens the thread. Many instances are found where a long thread has a very small satellite or none, and others where the thread of this chromosome is short and the satellite large. But winding or unwinding is probably not the whole explanation. There are many instances of constant difference in the size of the satellites in genomes of the same plant.

Fernandes (1936) speaks of the "nucleologenic region" of the sat-chromosome, including the thread and adjacent portions indefinitely, while Resende (1940) uses the terms "SAT-zone" for the nucleolus-producing area. He finds it to be in some cases, *e.g.*, *Encephalartos,* not a thread but a band containing chromomeres. All these observations go to show how variable is the character of the sat-thread. Its real nature is still uncertain, but the essential nucleolar producing body appears to be not on the thread or the satellite but at the tip of the chromosome proper.

THE PHYLOGENETIC SIGNIFICANCE OF NUCLEOLI

The material bearing on this subject is now too abundant to be treated fully here, but some of the more striking cases of variations in the satellites and nucleoli of related species may be cited, as well as certain aberrant species which require further investigation. Phylogenetic relations can be traced by combining the evidence from the nucleolar number with the sat. relations or secondary constrictions and the evidence from secondary pairing and general chromosome morphology. Very little has yet been done with animal cells in this field.

In *Lobelia* (Okuno, 1937), although the satellites and nucleoli have been inadequately studied, the three diploid species ($2n = 14$), *L. inflata, L. syphilitica* and *L. dresidensis,* are found to differ in their satellites, the first species having an equal pair, the second a pair of unequal size while in the third there is said to be only one sat-chromosome. An early case of the kind was recorded by S. Navashin (1927) in *Galtonia.* He found that the sat-pair of chro-

mosomes had a large and a small satellite, while in tetraploid cells there were two large and two small satellites attached to the nucleolus in prophase.

Woods (1937) has investigated the nucleolar conditions in a number of species and clones of *Tulipa*. In counting nucleolar numbers he has evidently failed to distinguish between true nucleoli and the transient matrix globules found in early telophate, as previously described. In roots, however, his average numbers per cell range from 3.29 to 5.44 in diploids $(2n = 24)$, from 6.32 to 7.91 in triploids and from 5.02 to 9.14 in tetraploids. In tulip *Adonis* $(2n = 24)$ six nucleoli were found in diakinesis and in root-tips. Four pairs of meiotic chromosomes were heteromorphic, only one member of each having a sat. In the fifth pair both members bore sats. In a triploid variety certain trivalent chromosomes were observed at diakinesis, each member of which bore a sat.

In *Crocus* (Pathak, 1940a) there appears to be a definite relation between sat-size and nucleolar size. In races of tomato, Lesley (1939) found three sizes of sat-chromosomes with corresponding differences in the size of the nucleoli. The longest chromosome had two attachments to the nucleolus, suggesting duplication of the nucleolar organizer region. A fuller study of the sats. in these chromosomes would be of interest. From such cases it would appear that the sat. may play something more than a passive part in the production of a nucleolus. M. Navashin (1926) described in *Crepis dioscoridis* $(2n = 8)$ a sat-pair in two sizes. By sowing 175 seedlings he found the conditions, (a) two large sats., (b) a large and a small, (c) two small, in the Mendelian ratio $43 : 90 : 42$. Medwedewa (1930) studied these races genetically and found segregation of the heterozygous type (as regards sat. size) in the ratio $1 : 2 : 1$, the race with large sats. being more vigorous than the other two. Frankhauser (1934) showed that diploid Triton embryos have two sat. chromosomes, triploids three. He also found in three diploid blastulae a constant difference in size of the two satellites.

The basic chromosome number in the genus *Crocus* is three. The relation of satellites and secondary constrictions to nucleoli can be seen from Table V (Pathak, 1940a).

From this Table many relationships can be seen. *C. Olivieri* $(2n = 6)$ has one pair of chromosomes with secondary constrictions producing nucleoli, but no satellites. *C. Salzmanii* $(2n = 24)$ has

TABLE V

	$2n$	No. sats.	No. sec. constr.	No. of telophase nucleoli and no. of chromosomes attached in prophase
Crocus Olivieri	6	..	2	2
C. zonatus	8	2	..	2
C. aerius var. Gray Lady	8	4	..	4
C. ochroleucus	10	2	..	2
C. susianus	12	2	..	2
C. pulchellus	12	4	..	4
C. speciosus var. *albus* ...	12	4	..	4
C. speciosus	18	6	..	6
C. Tomasinianus	16	2	..	2
C. Korolkowii	20	4	..	4
C. Salzmanii	24	2	2	4
C. sativus	24	3	..	3
C. sativus var. *Elewesii* ..	15	2	..	2
C. Tournefortii	30	2	..	2

one pair of sat-chromosomes and one pair with secondary constrictions, thus producing four nucleoli in telophase. The other species examined have only sat-chromosomes. *C. zonatus* $(2n = 8)$ has one pair of unequal satellites producing two nucleoli of unequal size. *C. aerius* var. Gray Lady, with the same chromosome number, has one pair of chromosomes (A) with unequal satellites and two others (B and C) of unequal length forming a heteromorphic pair with unequal satellites. They produce four nucleoli of different sizes. *C. susianus* and *C. pulchellus* have $2n = 12$, but the former has only two nucleoli of unequal sizes produced by a pair of chromosomes with sats. of unequal size. As it is a tetraploid species on the basis of three it has presumably lost a pair of sats. The latter species has four nucleoli of different size, having two pairs of sat-chromosomes with very small satellites, but one pair smaller than the other. Heitz (1926) regarded it as a diploid, but the presence of two pairs of nucleoli of different sizes indicates that it may be an allotetraploid. In "*C. speciosus* var. *albus,*" the third tetraploid species, the conditions of sats. and nucleoli are the same. It appears to be an autotetraploid in which one sat. has been translocated from the short to the long arm of a chromosome. *C. speciosus* with $2n = 18$ is a hexaploid, as confirmed by the presence of three pairs of sats. of two different sizes and six nucleoli of corre-

sponding size. Clearly this hexaploid form must have been derived from a tetraploid, and not the reverse, as the taxonomic names would imply. The chromosome morphology indicates that it is an allohexaploid derived from a cross between the original diploid and tetraploid forms followed by doubling of the chromosomes in the hybrid. In *C. Tomasinianus* $(2n = 16)$ with only one pair of sats. and nucleoli, several sats. have presumably been lost from the chromosomes. *C. Korolkowii* $(2n = 20)$ is like *C. pulchellus* $(2n = 12)$ in having two pairs of sats. and nucleoli of unequal size. A pair of sats. has probably been lost. *C. Salzmanii* and *C. sativus* with $2n = 24$ might be octoploid, hexaploid or even diploid in comparison with other species. The former has four nucleoli from two sats. and two secondary constrictions, while the latter has sats. on the long arm of three similar chromosomes which produce three nucleoli. This species is, however, neither an octoploid nor an autotriploid. *C. sativus* var. *Elwesii* $(2n = 15)$, having a heteromorphic pair of sat-chromosomes and two unequal nucleoli, is probably a hybrid between two 16-chromosome species in the diploid condition, such as *C. Tomasinianus,* one having a larger sat. on the long arm of a long chromosome, the other a smaller sat. on the short arm of a medium chromosome.

The three species with 10, 20 and 30 chromosomes, respectively, look like a polyploid series, but the number of nucleoli and the chromosome morphology shows that they are not all in one series. By further studies of more species it is evident that much may be learned regarding the relationship within the genus and some present taxonomic ideas can be corrected. From the original basic number three, other basic numbers, five, six, eight and ten, have evidently been derived.

The question of how satellites are lost is an important one. It appears that only one pair of nucleolar chromosomes with sats. or secondary constrictions is necessary for the life of the species cell. The frequent occurrence of apparently diploid species (see Table IV) having two pairs of nucleolar chromosomes shows that this condition, however it has arisen, is often very stable. There is evidence that in various rice varieties, however, one pair of nucleoli has been lost, probably by a mutation. This would mean the deletion, not only of the satellite and thread but also of the nucleolar organizer at the end of the chromosome (Ramanujam, 1937).

Warmke and Johansen (1935) found the haploid chromosome set of *Trillium ovatum* and *T. chloropetalum* to agree except that a sat. in the former is missing in the latter, but the nucleoli in these species require investigation. A process of loss has probably occurred in many species where the comparative chromosome numbers indicate tetraploidy but a single pair of nucleolar chromosomes exists, *e.g.*, several species of *Cassia* (Jacob, 1940*b*). It seems probable that a sat. can be lost also by gradual diminution until it is below the limits of visibility, and that further changes in the same direction will lead to its functional and structural elimination. Although the chromosomes of *Trillium* are among the largest in plants, yet some of their sats. are so minute that a slight diminution would render both sat. and thread invisible. Some of the sats. in *Crocus, Haworthia* and other genera are equally minute. Their loss may produce a gradual shortening of chromosome segments somewhat after the manner assumed by Delaunay (1926) in *Muscari.*

In *Narcissus,* unlike *Crocus,* there appears to be no relation between nucleolar size and sat-size (Sikka, 1940*b*). The range of sat. and nucleolar conditions appears to be wider than in *Crocus.* Heteromorphic pairs of sat. chromosomes, implying translocation of the sat., are common as is also reversal of a sat. chromosome segment, producing a lateral sat. When such a sat. and thread produces a nucleolus, the reversed portion of the chromosome must have included the nucleolar organizer at the proximal end of the chromosome. In *N. odorus* ($2n = 14$) one C chromosome has a sat. on the short arm and one D chromosome has a sat. on its long arm. They produce two nucleoli of unequal size. In *N. poeticus* ($2n = 14$) one D and one F chromosome form a heteromorphic sat. pair. There are secondary constrictions in the BB chromosome pair, but they do not form nucleoli. Sâto (1938) found a triploid race with three nucleoli. In *N. gracilis,* a third diploid species, an F chromosome has a sat. on its long arm, as in *N. poeticus,* but the other sat. is on the short arm of a G chromosome. Translocations between all the chromosomes have probably been frequent.

Probably seven is the basic number in the genus *Narcissus,* from which six and five have later been derived. The evidence from the satellites and nucleoli indicates that five is the cardinal number in *N. Tazetta,* it being hexaploid on this basis. A Mediterranean variety of this species with $2n = 21$ is probably a hypertetraploid

since it has four sats. and four nucleoli. Sâto (1938) found four sats. in var. *papyraceus* with $2n = 22$. Another variety with $2n = 20$ has only two nucleoli and sats. although there are six secondary constrictions which are non-nucleolar. Probably a pair of organizers has been lost through mutation or amphiplasty. A third, var. *canaliculatus* ($2n = 29$), appears to be hypohexaploid as it has six nucleoli, four produced at sats. and two at secondary constrictions. That *N. pseudonarcissus* has a basic number seven is also indicated by the nucleoli. Thus var. *Emperor* ($2n = 21$) has three sat. chromosomes and produces three nucleoli, one large and two small. Var. *Victoria* ($2n = 22$) also produces three nucleoli of very different size, while var. *King Alfred* ($2n = 28$) produces four but is not an autotetraploid.

Another feature of much interest which is clearly shown in *Narcissus* is the time element in relation to nucleolar production. In this genus, at least the first pair of nucleoli can be seen with the Feulgen-light green stain to arise on particular chromosomes in anaphase, before a nuclear membrane is formed. In *N. pseudonarcissus* var. *Victoria*, which is hyperallotriploid ($2n = 22$), having two long chromosomes with sats. on the long arm and a small chromosome bearing a sat. on the short arm, two nucleoli appear in anaphase but grow at unequal rates while a third appears later, in telophase, and remains very small. From this and other evidence there is clearly competition between organizers for the matrix material from which the nucleoli are constructed. Some nucleoli also begin to grow earlier than others and they may grow at different rates.

In the genus *Cassia* (Jacob, 1940*b*) *C. auriculata* is diploid with 14 chromosomes and has one pair of nucleoli produced by the B pair of sat-chromosomes. The A pair is longer and is like a chromosome in which the sat. end has been inverted. It has a long constriction but is non-nucleolar. If this is a true case of inversion the nucleolar organizer has been lost. The G chromosome is shortest and has a non-nucleolar secondary constriction. In one plant this chromosome was duplicated, giving a plant with 16 chromosomes. Two other species of *Cassia* have $4n = 28$ chromosomes and four nucleoli, while four more have 28 chromosomes but only two nucleoli, presumably a derived condition. *Clitoria ternata* with $2n = 16$ chromosomes is tetraploid, having four sats. and nucleoli,

in addition to two pairs of non-nucleolar secondary constrictions. Two lateral sats. due to translocation were found in one cell. *Poinciana regia* with $2n = 28$ chromosomes appears to be heptaploid as it has seven nucleoli. This and other evidence indicates that four is the basic chromosome number in the Leguminosae. Thus Iyengar (1939) finds that *Cicer arietinum* has 16 chromosomes, four with sats., these four attached to the nucleolus in somatic prophase, while *C. soongaricum* with 14 chromosomes also has four sats.

In cereals the relations of nucleoli to chromosomes are particularly clear. They enable the different genomes to be traced. Much evidence on this subject has already been obtained by study of the nucleoli and the chromosome morphology (Pathak, 1940*c*) as well as from crosses. *Triticum monococcum* ($2n = 14$) has two pairs of sat-chromosomes with larger and smaller sats. and four nucleoli also of two sizes. In morphology the seven chromosomes fall into five types, BD and FG being alike. Occasional quadrivalents are also seen in meiosis. All this indicates that in *Triticum*, as in *Oryza*, five was the original basic number, from which seven is derived. This was probably not a simple process of reduplication, however, because while BD and FG are duplicates, C and E, the two sat-chromosomes, are unlike. In *T. aegilopoides* the chromosomes and nucleoli are in the same condition as in *T. monococcum*. The three tetraploid species, *T. durum, Aegilops cylindrica* and *Aeg. ovata,* all agree in having four nucleoli (two size pairs) produced on a pair of sat-chromosomes and a pair with secondary constrictions. The hexaploid species, *T. vulgare, T. spelta* and *Aeg. crassa,* each have three pairs of nucleoli of different sizes produced by two pairs of sat-chromosomes and one pair with secondary constrictions. Thus the various genomes of hexaploid wheat can be identified and further study may make it possible to determine each of the six genomes which were finally combined to produce *T. vulgare.*

The cytological study of numerous species of the widespread genus *Allium* (Mensinkai, 1939*b*) leads to the conclusion that eight is the original haploid number in this genus, from which seven and nine have been derived. Levan (1935), however, regards seven as the primitive number. He has compared the sats. and secondarily constricted chromosomes in a number of species, but without reference to their nucleoli. Allopolyploidy based on the numbers

seven, eight and nine has frequently occurred. The derivation of nine from eight chromosomes has been explained (Mensinkai, 1939*b*) through breakage of the centromere of a V- or J-shaped chromosome, followed by reciprocal translocation. *A. sativum* and *A. cepa* ($2n = 16$) are secondarily balanced diploids with two pairs of non-homologous nucleolar chromosomes. Eight other species of *Allium* with $2n = 16$ are ordinary diploids with one pair of sats. or secondary constrictions and two nucleoli. *A. sativum* has no sats. and its condition might have arisen through double non-disjunction of a nucleolar pair and another pair of chromosomes. The related *A. Cepa* has one sat-pair and is an inversion heterozygote.

There is evidence of frequent inversions, translocations and interchanges in this genus. For instance, Taylor (1926) found tandem sats. in a plant of *A. Cepa,* a condition which must have arisen by interchange. In *A. darwasicum* Mensinkai found one metaphase plate with tandem sats. This probably arose through a double interchange or crossover between the two homologous sat-chromosomes. Srinath (1939) found in *Calceolaria Allardi* one long chromosome with tandem satellites as a rare condition in somatic nuclei, which probably arose through a double interchange with its mate. Sâto (1939) describes tandem sats. in *Tricyrtis latifolia.* Such an exchange between two homologues could take place when two sat. chromosomes are attached near together to the nucleolus and their threads become entangled. Jacob (1941) has described a heteromorphic pair with one terminal and one lateral sat., in roottips of *Gossypium arboreum* and *G. herbaceum.*

In *Allium amplectens* ($2n = 14$) Mensinkai (1939*b*) finds three pairs of sats. and six nuclei, a situation which requires further study. Levan (1940) finds in this Californian species an asynaptic type. He found no sats., yet he figures a chromosome terminally attached to the nucleolus. In the resting nucleus he finds many nucleoli, but this is apparently the evanescent stage of the telophase globules already described. *A. Bidwelliae* ($2n = 28$), also from California, has five pairs of secondary constrictions and five pairs of nucleoli. This unique condition in an allotetraploid requires fuller investigation. *A. margaritaceum* occurs in a diploid ($2n = 16$) and a tetraploid form. The former has a sat. pair, while the latter is apparently an allotetraploid as it has one sat. pair, and one

pair with secondary constrictions. *A. senescens* from Siberia and *A. giganteum* from the Himalayas are hexaploids with 48 chromosomes, three pairs of subterminal constrictions and six nucleoli. A diploid form of *A. giganteum* is known (Levan, 1935). *A. ascalonicum* has a heteromorphic pair of sat-chromosomes, one sat. being very large and the other extremely minute.

In the genus *Calceolaria* Srinath (1939) examined many species and hybrids. Among ten diploid species ($2n = 18$) seven had two nucleoli, the other three having a pair of sats., a pair of secondary constrictions and four nucleoli. Six species and eleven garden hybrids were tetraploid. Five of these species and ten hybrids had four nucleoli. In one $4n$ species (*C. Pavonii*) and one hybrid eight chromosomes were attached to the nucleolus in somatic prophase. How such a condition could arise requires investigation. *C. mexicana* ($2n = 60$) is a naturalized species which has spread all over India (Srinath, 1940) and has probably undergone some chromosome evolution there, giving a derived octoploid condition. Only two bivalents are attached to the nucleolus in the pollen mother cells, however, but hexavalent chromosomes are found, so that several sat-chromosomes must have been lost.

The Phalarideae are a group nearly related to the Oryzeae. Parthasarathy (1939) finds that three diploid species of *Phalaris* had $2n = 14$ chromosomes, two others $2n = 12$, all five having two sat-chromosomes. Three other species of *Phalaris* were tetraploid ($2n = 28$). *P. arundinacea,* having four sats. and four nucleoli, is probably an autotetraploid. *P. tuberosa* has four nucleoli but the sats. could not be seen. *P. minor,* having two nucleoli and two sats., and forming 14 bivalents in meiosis, is an allotetraploid in which a pair of nucleoli has been lost. In the genus *Ehrharta* the chromosomes are very small, like those of rice, and no sat. was visible. *E. erecta* ($2n = 24$) has in meiosis two bivalents attached to the nucleolus, indicating a secondary tetraploid as in rice. In *E. calycina* and *E. longiflora* ($2n = 48$) only four chromosomes are attached to the nucleolus in somatic prophase although these are apparently secondary octoploids. In *Oryza coarctata* ($2n = 48$) there were similarly only four nucleoli (Parthasarathy, 1938), but *O. Eichingeri* and *O. sylvestris,* with the same chromosome number, have eight chromosomes attached to the prophase nuclei, thus showing their octoploid character (Pathak, 1940*c*). *O. minuta*

$(2n = 48)$, which is probably an amphidiploid from *O. officinalis* × *O. sativa* (Nandi, 1936), has four bivalents attached to the nucleolus in meiosis. Besides *Ehrharta* and *Oryza,* the nearly related *Leersia* has $2n = 48$ chromosomes (Ramanujam, 1938) while *Hygrorhiza aristata* has $2n = 24$. By contrast, *Zizania* $(2n = 30)$ and *Lygaeum spartum* $(2n = 40)$ are high polyploids of the original basic number five.

In *Crataeva religiosa* (Raghavan and Venkatasubban, 1939) $n = 13$ and the secondary pairing in meiosis, 2 (3) + 2 (2) + 3 (1), indicates that the basic number is seven. Its secondary polyploid nature is confirmed by the attachment of two bivalents to the nucleolus in diakinesis and four chromosomes to the nucleolus in somatic prophase.

In various genera such as *Oryza* and *Trillium* with very small sats. the chromosomes have been described as terminally attached to the nucleolus in prophase. The demonstration of sats. in these plants makes it probable that the terminal position is a secondary one owing to the minuteness of the sat. In Haga's (1934) study of *Paris* and *Trillium* he shows five types of chromosomes, A, B, C, D, E. He finds diploid and octoploid species of Paris and $2n$ and $4n$ species of *Trillium*. His observations of the sats. are inconclusive. He shows a large sat. on the D chromosome in *Paris* but states that it is absent from this chromosome in *Trillium*. Resende (1937) states that in *T. erectum* there is a relatively large sat. on the long arm of the B chromosome, as well as minute ones on the A, D and E chromosomes, thus making four pairs of sats. in a plant with only five pairs of chromosomes. He finds the same condition in *T. longiflorum* except that all the sats. are minute. The existence of so many sats. in diploid *Trillium* is very improbable. In *T. ovatum* Resende found only two sats. In *T. sessile,* Mensinkai (1939*b*) observed a minute sat. on the long arm of one C and one D chromosome. Matsuura (1938), in a study of *T. kamtschaticum,* was unable to find the minute sats. and concluded that the A chromosome produces a nucleolus terminally at both ends while the E chromosome produces another terminal nucleolus on its shorter arm. He finds nucleoli numbering up to nine in a cell, which suggests that the nucleolar organizer is absent. Or perhaps he included the evanescent telophase stage in which droplets are formed from the chromosome matrix and have not yet been

attracted into the true nucleoli. The whole subject of sats. in *Trillium* requires re-investigation, with more attention to the nucleoli. It is probable that the minute sats. and the organizers have become non-functional in some species.

The study of sats. and nucleoli in *Brassica* (Sikka, 1940*c*) reveals many interesting features, as shown in Table VI. In this genus, where the chromosomes are very small, the satellites may be missed, but the number of bivalents attached to the nucleolus in meiotic prophase is equally significant.

TABLE VI

	$2n$	Sats.	Nucleoli	Remarks
Brassica nigra ..	16	4	4	Perhaps hypoallotetraploid.
B. oleracea	18	2	2	1 bivalent attached to nucleolus in meiosis.
B. rapa	20	2	2	
B. campestris ...	20	2	2	
B. trilocularis ..	20	2	2	1 bivalent attached to nucleolus in meiosis.
B. Tournefortii .	20	2	2	
B. monensis	24	..	..	1 bivalent attached to nucleolus in meiosis.
B. juncea	36	6	6	
B. rugosa	38	..	4	
B. napus	38	..	4	
B. sinapistrum ..	18			
B. Wrightii	24			
B. cheiranthus ..	48			

From this table it will be seen that although *B. nigra,* which has the lowest known chromosome number in the genus, has four nucleoli, several species with higher chromosome numbers have only two. They have probably lost a pair of nucleolar organizers. The maximum secondary pairing was $1(4) + 1(3) + 2(2) + 1(1)$, found in *B. monensis,* and $4(2) + 1(1)$, found in *B. sinapistrum,* indicating that five is the basic number in the genus. This is supported by the fact that three species of *Erucastrum,* in the same subtribe, have 32 chromosomes, *i.e.,* they are probably hexaploids in which two chromosomes have been reduplicated. This can be tested by a study of their nucleoli. Further, in the genus *Crambe* of a related subtribe the $2n$ numbers are 30, 60, 90, 120. The hexaploid number 30 has therefore been the starting point for this genus.

B. nigra is probably an allotetraploid which has lost four of its smallest chromosomes. By a study of the meiotic pairing in their hybrids as well as the chromosome morphology and the nucleoli, Sikka has shown that *B. juncea,* with six nucleoli and 36 chromosomes, is probably an amphidiploid of *B. nigra* and *B. campestris. B. napus* is similarly an amphidiploid of *B. oleracea* and *B. campestris, B. carinata* $(2n = 34)$ an amphidiploid of *B. nigra* and *B. oleracea,* while *B. rugosa,* with four nucleoli, is also an amphidiploid. The nucleolar numbers thus play an essential part in showing the prevalence of amphidiploidy in the genus *Brassica.*

In the Amaryllidaceae (Sâto, 1938, 1939) very different conditions obtain. Where the size of the sats. is extremely small, as they frequently are, it is essential that their number be checked by examination of the nucleoli. Secondary (nucleolar) constrictions are also easily overlooked. Sâto has made a study of the chromosome morphology of the species in many genera. In *Haemanthus albiflos* $(2n = 16)$, for instance, he finds two sats. on the short arm of a long pair of chromosomes and four sats. on the long arm of medium chromosomes, but this very high number of sats. has not been checked by observing the nucleoli. In *Griffinia Blumenavia* $(2n = 77)$, believed to be heptaploid, neither sats. nor nucleoli were seen. *Clivia nobilis* $(2n = 44)$ has four nucleoli and probably four sats. and *Galanthus nivalis* $(2n = 24)$ also has four sats. *Leucojum autumnale* $(2n = 14)$ has four sats. and nucleoli, a further addition to the number of diploids in this stable condition. In the genus *Agave,* where $2n = 60$ (10 long and 50 short), 90, 120, 150, 180, there is no reference to either sats. or nucleoli. Sâto finds nonnucleolar constrictions in *Zephyranthes, Cyrtanthus, Habranthus* and *Narcissus.*

In the genus *Tricyrtis,* Sâto (1939) finds five species with $2n = 26$ chromosomes. Two of them have six sats. and six nucleoli, two species and a variety have four sats. while another species has only two. In somatic prophase he finds four to ten chromosomes attached terminally to the nucleolus and calls them all nucleolar chromosomes. The attachment of the non-nucleolar chromosomes requires explanation. Perhaps there are more than four nucleoli and some of the sats. have been missed because of their minute size. Coleman (1940) states regarding *Veltheimia viridifolia* $(2n = 40)$ that "satellites appear to be absent." In the pollen grain mitosis

one of the smallest chromosomes is always attached terminally to the nucleolus in prophase.   As *Veltheimia* belongs to a group of genera in some of which the sat. has become very minute, it is probable that in this case the sat. and the thread have really disappeared, the nucleolus being formed terminally by the nucleolar organizer. We formerly suggested that in rice the chromosomes which were attached terminally to the nucleolus were without sats., but we afterwards demonstrated their presence in these minute chromosomes.   On the other hand, the chromosomes of *Veltheimia* are large.   It is probable that the very minute sats. in some genera such as *Trillium* are of the nature of vestigial structures—nonfunctional and on the verge of disappearance.   When the sat. is large the nucleolus is necessarily lateral in origin, but when it becomes very minute the nucleolus may become essentially terminal by pushing the sat. aside even though it has not entirely disappeared. On this basis, terminally attached chromosomes may be expected in genera where the sats. have become very minute.   In the liverwort, *Pallavicinia lyellii*. Walcott (1939) describes one nucleolar chromosome in the gametophyte nuclei and two in the sporophyte. No sat. was found, and these chromosomes were each terminally attached to a nucleolus, but as the writer saw "apparent satellites on two prophase chromosomes" the absence of sats. cannot be regarded as proved in this case.

The genus *Paeonia* (Sinotô, 1938) has some interesting features.   Several species have $2n = 10$ chromosomes but eight very small sats., a very high number for plants which cannot be octoploid or even tetraploid.   Different cultivated varieties of *P. albiflora* have eight, seven or six nucleoli and sats., indicating that some of the extra ones are being lost.   The condition of the nucleoli in this genus would repay further study.

In *Datura stramonium,* Satina, Bergner and Blakeslee (1941) have shown that the 12 pairs of chromosomes can all be identified by their morphology.   Six pairs are found to have sats. and two other pairs have secondary constrictions of peculiar character, but the relation to nucleoli has not yet been investigated.   Two of the interchange chromosomes, $10 \cdot 24$ and $12 \cdot 21$, have a sat. at both ends.

The genus *Carex* and other Cyperaceae are well known for their long aneuploid series of chromosome numbers (Heilborn, 1939).

The known haploid numbers in *Carex* species are 6, 8, 9, 12, 13, 15–19, 22–43, 54 and 56, or 34 different *n* numbers. Allopolyploidy appears to be lacking in the genus, but cases of autopolyploidy occur. For the identification of such cases the nucleoli as well as the chromosome morphology are necessary. Tanaka (1939, 1940) finds in *C. siderosticta* ($2n = 12$) one pair of sats. while a $4n$ form has four sats. Similarly, *C. lasiolepis* ($2n = 16$) and *C. oxyandra* ($2n = 18$) have one pair, while in *C. japonica* ($2n = 62$) there are two pairs. More attention to sizes and numbers of sats. and nucleoli, combined with the study of secondary pairing in meiosis, will doubtless help in determining relationships of the species in this group.

<center>NUCLEOLAR BUDDING</center>

It has often been denied that true budding of nucleoli occurs in plants cells. In rice it has been shown that zygotene pairing of the nucleolar chromosomes will obviously drag the homologous nucleoli together as the threads pair, so that the paired threads lie between the nucleoli, thus generally preventing them from touching and fusing (Nandi, 1937). This clearly is not budding but pairing. On the other hand, the larger nucleoli of pollen mother cells of rice in synizesis form a bud which "is always at the point of attachment of a terminal chromosome knob." The bud or protuberance "grows until the two nucleoli attain the same size at early diakinesis by transfer of material from the larger to the smaller." Such a process probably means the separation of substances which are mingled in the original nucleolus. Selim (1930) found in rice varieties that the primary nucleolus of the pollen mother cell buds off a secondary nucleolus which is paler staining and disappears earlier in meiotic prophase than the primary one. He concluded that this process of budding separated two different materials, the secondary nucleolus contributing to the chromosomes while the primary may contribute to the spindle.

Van Dillewijn (1940) describes in the pollen mother cells of *Populus* various cases of nucleolar budding, as well as the true pairing of nucleoli attached to homologous zygotene threads. He cites several other instances from the literature.

The cytology of *Antirrhinum* is much like that of maize. The eight chromosome pairs were shown (Ernst, 1939) to be all mor-

phologically different and they appear to have knobs or deeply chromatic areas on the pachytene threads, resembling those of maize. In a particular strain, Resende (1940) finds three nucleoli in the pachytene stage, one of them much larger than the other two. The three sat. chromosomes have a knob where they join the nucleolus. Particular chromomeres and deep staining bodies can be identified at loci in each chromosome. (Four nucleoli are recorded in two nuclei without explanation.) An observation of special interest regarding the nucleoli in the pollen mother cells, which confirms the earlier observations and conclusions with regard to the nucleoli in rice, is that the large nucleolus forms a bud of secondary nucleolar substance which arises at the point of attachment of this nucleolus to the pachytene chromosome, *i.e.*, at the nucleolar body. It is paler staining than the original nucleolus and in its origin a different substance is evidently separating out. This budding was not seen in telophase, nor in somatic prophase.

In a haploid *Antirrhinum* pachytene pairing of the eight chromosomes takes place and multivalents are frequently formed (Ernst, 1940), from which it is concluded that the original chromosome number was not eight but seven or six. Even this makes it difficult to account for three unlike sat-pairs in a plant with only four pairs of chromosomes. Probably in some plants the nucleolar functions or substances have become differentiated, so that two or three nucleolar pairs produce different substances which are all produced at one pair of chromosome loci in other plants. It has already been pointed out that the widespread occurrence of diploid plants with two unlike pairs of nucleoli may indicate, not an allo-tetraploid condition but one in which the functions of the two nucleolar pairs have become separated.

The budding process in which two nucleolar substances seem to separate in the nuclei of pollen mother cells resembles in some respects the separation into basophilic and oxyphilic portions of the nucleoli in animal oocytes in connection with yolk formation and in animal eggs. They probably both represent the progress of certain chemical changes taking place in the nucleus.

NUCLEOLI IN SALIVARY GLAND CELLS

The modern work on the salivary gland chromosomes of insects has included various studies of the nucleoli. Kaufmann (1934)

made a study of the somatic mitoses in ganglion cells of *Drosophila melanogaster* in which it was shown that the X-chromosome has a secondary constriction and the Y-chromosome a large sat. on its short arm. These are both associated with the development of nucleoli, the sex chromosomes being heteropycnotic. He has since (1938) shown that the heterochromatic nucleolus organizing region in the salivary chromosomes, involving certain bands of the X, can be translocated to other chromosomes. King and Beams (1934), in a study of the salivary chromosomes of *Chironomus,* found four pairs of chromosomes synaptically paired. Chromosome *a* produced a ring or small nucleolus (Balbiani's ring) at one locus while the larger plasmosome was similarly produced at a definite locus of chromosome *b*. In *Chironomus Thummi,* Bauer (1935) found that the structural features of the four (paired) chromosomes could be seen in the living condition. By mounting in paraffin oil, the large nucleolus was seen attached to the small chromosome IV. This chromosome was also the bearer of some small granular nucleolus-like masses, which are figured but whose nature is not clear, as well as Balbiani's ring. The left end of this chromosome also fans out into a loose terminal structure which is regarded as nucleolar. This condition, in which at least two nucleoli are produced from different loci of the same chromosome, appears to be unique. The larger chromosomes produce in a few nuclei droplets or Nebennucleoli at definite loci along their banded length. They arise periodically and lie free in the nuclear sap. These are the bodies described by Faussek (1913) as "argentophile granules." In living cells they can be seen localized at particular bands, those that break free showing Brownian movement.

All these observations go to show that various substances in the nucleus are secreted from the chromosomes or produced by them at particular loci by interaction with other substances. This considerably widens the ordinary conception of the nucleoli and enables us to see in some measure how the chromosomes produce various substances, some of which acquire structural validity in the nucleus while others are shortly utilized in the cell metabolism and so disappear. When these various substances can be chemically identified we shall know more about nuclear metabolism in its general and special features.

Alverdes (1912) made various observations on the nucleoli in

salivary gland nuclei and refers to observations of several other workers, but he came to no definite conclusions about them. Van Herwerden (1911) concluded from various microchemical reactions that the nucleolus did not contain chromatin, as had been previously affirmed. Balbiani (1881), in his original paper, concluded that the nucleoli differed manifestly from the "cordon intranucléaire" in their morphological and chemical characters.

Poulson and Metz (1938) have investigated particularly the nucleolus-forming regions of the salivary chromosomes in two species of *Chironomus* and in *Sciara ocellaris*. In *Chironomus* they found, like Bauer, that the large nucleolus, which is so large that it is much distorted and flattened out in a smear preparation, is associated with chromosome IV. It of course represents a homologous pair of nucleoli belonging to the two closely synapsed chromosomes. The banded structure of the chromosome is modified by swelling and dispersion in this region and is heterochromatic. The smaller nucleolus (Balbiani's ring), also representing a pair, is on the same chromosome, and the banded structure is less distributed at this point. The bands are extended as a branching network into the nucleolus. The Nebennucleoli were seen as small achromatic spheres at various points along the chromosomes. In *Sciara* no true nucleoli were found, but "puffs" or "bulbs," which are expansions of the chromosomes, appear to contain nucleolar material, which is therefore inside rather than outside the polytene chromosomes. The puffs all act together in becoming larger or smaller, as though they were connected inside the chromosome. Their condition probably depends on physiological rather than genetical conditions. The two pairs of nucleoli in *Chironomus,* which has only four pairs of chromosomes, is another clear case where there can hardly be allotetraploidy. They presumably represent the major differentiation in nucleolar functioning. The Nebennucleoli may be secretions from the chromosomes, occurring at particular loci. Although not yet seen in other types of chromosomes, they probably occur as interaction products.

SEX CHROMOSOMES AND NUCLEOLI

That the satellited or nucleolar chromosomes are frequently the sex chromosomes has been noted by several investigators. Gates (1939) pointed out that this condition appears to be general,

though not quite universal, not only in Diptera b t also in liverworts and mosses. When there is a second pair of nucleoli they will probably be on a pair of autosomes. The freq ent occurrence of the condition in which the nucleoli are produced on the sex chromosomes would argue for some advantage in this condition. Corey (1940) suggests that where this relation exists there has been translocation from an autosome to the X. Apart from the salivary gland chromosomes of *Chironomus,* two pairs of nucleoli never appear to be formed on one pair of chromosomes. Such a condition has apparently never been found in plants.

Kawaguchi (1938) finds that in diploid silkworm spermatogonia there are two nucleoli, in triploid three and in tetraploid four. But the oogonia have one nucleolus in diploids, two in triploids and tetraploids. This agrees with the known number of Z-chromosomes, so the Z and not the W must be the sat-chromosome.

In *Drosophila melanogaster,* Kaufmann (1937) showed that the X-chromosomes are attached in prophase to the nucleolus which they produce. The same situation is described in several other species of *Drosophila. D. ananassae,* however, is an exception. Kaufmann (1937) showed that in this species the X-chromosome is not nucleolar, but a pair of autosomes with satellites is attached to the nucleolus. But the Y has a sat. and is also attached to the nucleolus in the cells of the male. This condition has probably been produced, as Kaufmann suggests, by translocation between the X and an autosome.

Tatuno (1941), in a recent further study of liverwort chromosomes, finds, for instance, that in *Marchantia polymorpha* the X and Y are heteropycnotic and a pair of autosomes have a sat., while in *M. radiata* an undifferentiated heteropycnotic pair is attached to the nucleolus. All the sex chromosomes are more or less heteropycnotic and a pair of autosomes is frequently also heteropycnotic, but little attention has been paid to their attachment to the nucleolus. There is evidently some relation between heteropycnosis and the sat. (nucleolar) chromosomes, as well as the sex chromosomes.

No doubt a survey of the cytological literature would lead to the recognition of many unrecorded cases in which the sex chromosomes are also the nucleolar chromosomes. For instance, Blackman (1905), in a study of myriapod spermatogenesis, gives several figures showing the "plasmosome with the accessory chromo-

some closely apposed to one side, and the other chromosomes lying freely in the nucleus." This must mean that the sex chromosome has produced the nucleolus and that it has either a sat. or a secondary constriction, unless the latter is too small to be visible.

SUMMARY AND CONCLUSIONS

Early observations and studies of the nucleolus by Fontana, Wagner, Valentin, Schleiden, Zacharias, Montgomery and others are cited.

Various cases of nucleolar extrusion from animal eggs in yolk formation and from other tissues in connection with secretion are described, proving that nucleoli or portions of nucleoli pass through the nuclear membrane into the cytoplasm, frequently as solid bodies, during these processes.

The use of the Feulgen-light green stain for the study of nucleoli in smears and sections is briefly described.

As regards the lower organisms, the lower Cyanophyceae do not have a nucleolus. In such algae as *Spirogyra* and *Lomentaria* the chromosomes migrate into the nucleolus in prophase and the rest of the mitosis is *intranucleolar*. In Ciliates the nucleoli remain as elongated structures surrounding portions of certain chromosomes and were formerly known as macrochromosomes.

The chemical composition of nucleoli has recently been investigated, using the ultra-violet absorption spectrum method. Ribonucleic acids are thus shown to be present, giving a characteristic absorption curve due to the conjugated double bonds of the pyrimidine rings. Inconclusive histological tests of root-tip nucleoli indicate the presence of lipoids; but recent tests of the nucleolus in fresh starfish and other invertebrate eggs (Gates, 1942) with Feulgen (without hydrolysis) show that it remains colorless, indicating the absence of phospholipids with free aldehyde groups from this body. Observations of fresh eggs with an immersion lens indicate the presence of two immiscible substances. The ribonucleic acid in nucleoli may be derived from the chromosome sheath or matrix. There is evidence that nucleoli also contain phosphorus, probably glutathione and also proenzymes.

Various measurements of nucleoli under different conditions show that they grow exceptionally large if nuclear division is inhibited. In regeneration of mosses and liverworts both nuclei and

nucleoli increase in size, but the latter in a higher proportion than the former. Meristem nucleoli are conspicuously large, the ratio $\frac{\text{nuclear vol.}}{\text{nucleolar vol.}}$ being *ca.* 16: 1 in meristems, 30: 1 in rootcap cells and 40: 1 in cells which are fully differentiated. The size of the nucleoli appears to be much influenced by nutrition. They become smaller in leaves kept in darkness and are found to show normally a diurnal size variation of 26%—50%, presumably due to variation in their sugar storage content.

Comparative measurements of nucleolar volume in polyploids, where several nucleoli fuse into one, show that the single nucleolus of a tetraploid or hexaploid is larger than the sum of its component nucleoli. This may be due to the inclusion of karyolymph between the fusing nucleoli. In hexaploid wheat the volume of nucleolar material is greater than in tetraploid wheat. On the other hand, in an autotriploid rice there was no increase in nucleolar volume over the diploid. Similarly, in petunia polyploids there was no correlation between size of nucleolus and number of chromosomes. But in the root-tip nuclei of diploid and haploid rice plants the relative nucleolar volume was over 2: 1 and in a crocus plant with a tetraploid root sector the nucleolar volume was approximately twice that in the diploid portion of the root.

The discovery of satellites by S. Navashim, of the nucleolar body in pollen mother cells by Latter and of the nucleolar organizer by McClintock mark important steps in the modern study of how nucleoli arise in the nucleus. The present analysis shows that the nucleolar body of Latter has been observed also by Carothers in the meiotic nuclei of male insects where they were formerly called chromomere vesicles, and probably in human spermatogenesis. While the nucleolar organizer is the point in the chromosome from which a nucleolus arises and is not visible as a separate entity, the nucleolar body is a differently staining, (but non-chromatic) part of the nucleolus at the point of attachment of the nucleolus.

Each nucleus in the cells of nearly all organisms contains at least one pair of nucleolar chromosomes, each of which produces a nucleolus. Generally this is a chromosome with a satellite and the nucleolus appears to arise, not from the satellite or its thread but from the tip of the chromosome body where the nucleolar organizer is situated. The thread itself is sometimes below the limits of

visibility, but it frequently stains with Feulgen. The satellite ranges in size from at or below the limits of microscopic resolution to a globular body as wide as the chromosome. Variations in size occur in genera such as *Allium,* which, according to one hypothesis, is due to unrolling of the satellite, correspondingly lengthening the thread. On the other hand, the thread may be a spiral of a lower order than the chromonemata of the chromosome. In cases of amphiplasty it probably fails to be despiralized because the production of a nucleolus is suppressed by the competition of other organizers. The same nucleus may contain chromosome pairs with satellites of constantly different sizes, or the satellites may even be of unequal size in a heteromorphic pair of chromosomes. In certain cases, there appears to be a correlation between nucleolar size and sat. size, but in many plants no such relation seems to exist. Occasionally tandem sats. are produced, probably by interchange between the two sat. chromosomes. Observations of the nucleoli in such cases would determine whether the sat. or the thread has any part in the production of a nucleolus.

Nucleoli may also be produced at secondary constrictions in a chromosome, the primary constriction being the spindle fibre attachment. Apparently this differs from a satellite constriction merely in that the nucleolar organizer is situated farther back from the end of the chromosome, although there are a few cases where a secondary constriction appears to have arisen by reversal of the terminal segment containing a satellite. In a few plants, such as rye $(2n = 14)$, there is a single pair of chromosomes with secondary constrictions, but no satellite. In certain species of *Narcissus* and other genera non-nucleolar secondary constrictions occur as constant features at certain loci of particular chromosomes.

In telophase, each nucleolus arises on a sat. chromosome or at a secondary constriction. The process, as described in detail, is essentially the same in plant and animal cells. The telophase chromosome consists of two strands, each with a terminal sat. With the Feulgen-light green stain, the origin of the nucleolus can be determined as two minute green granules, one on each strand, at the base of the sat-thread. As they grow they shortly merge to form the nucleolus, which gradually grows to full size.

Primary or secondary polyploids have a corresponding increase in the number of nucleoli, which can be counted in early telophase

before they have begun to fuse.   Thus tetraploid whe ts have four
nucleoli and hexaploids six.   At first it appeared tl at a diploid
would have only one pair of nucleolar chromosomes, and that any
plant with two pairs must be a secondary tetraploid or at any rate
that some of its chromosomes had undergone some f rm of duplica-
tion.   So many cases are now known, however, in which whole
genera of diploid species have four nucleoli (two pairs) that the
great stability of this condition may require some other explana-
tion.   To consider only species with 14 chromosomes and four
nucleoli, this condition exists in the genera *Oenothera, Gaura, Gas-
teria, Aloe, Haworthia, Hordeum* and others (see Table IV),
often in many and sometimes in all of the species.   Frequently in
such cases one pair of chromosomes bears sats. and the other sec-
ondary constrictions; and the two pairs of nucleoli frequently
differ constantly in size.   The functions and composition of these
two pairs may therefore be differentiated, both being required for
the life of the cell, whereas in diploids with only one pair of nucleoli
the two substances would be produced in the same nucleolus.   This
problem still requires solution.   The view here suggested is per-
haps supported by the observation (Gates, 1942) that in the living
egg of the starfish and other marine invertebrates the single nucleo-
lus under an immersion lens generally contains two apparently
immiscible substances, one enclosed within the other.   In all such
eggs before maturation (meiosis) the single nucleolus presumably
represents a fused pair.   In any case, it seems clear that in such a
plant species as *Oryza sativa,* which is shown by several lines of
evidence to be a secondary tetraploid in which the basic number
has changed from five to 12, the two pairs of nucleoli which are
present in many varieties are not functionally differentiated, since
some varieties function perfectly with only one pair, having appar-
ently lost the other pair through a mutation.

    The history of the nucleoli in each mitotic cycle can be stated as
follows: in telophase of mitosis they arise at fixed loci on certain
chromosomes, sometimes beginning as early as anaphase.   In
growth they use the material of the chromosome sheath or matrix,
which leaves the chromonemata in early telophase and in several
genera is transiently seen in the form of numerous droplets scat-
tered throughout the nucleus.   These are used up as the true nu-
cleoli grow at their loci.   This evanescent stage has frequently

led investigators to the mistaken view that nucleolar numbers have no significance. The true nucleoli will fuse like droplets, however, whenever their growth or the movements of the chromosomes in the nucleus brings them into contact. Before prophase they have generally all fused into one, to which are attached at their points of origin, with or without satellites, the chromosomes that produced them. During prophase the nucleolus probably contributes part of its substance to the formation of the sheaths of the chromosomes. The rest is thrown out into the cytoplasm in metaphase, where it disappears. As the original material of the nucleoli has been closely in touch with the genic material, this fact and its later discharge into the cytoplasm may be of genetic and developmental significance. Some of the nucleolar content may be merely a source of energy in the cell.

The growth of the nucleolus at the organizer could be accounted for by assuming a higher electric charge at that point and a monomolecular layer of protein surrounding the growing body.

Comparative studies of the nucleoli and sats. in related species and genera, when combined with other lines of cytological evidence, such as secondary meiotic pairing and chromosome morphology, throw much light on nuclear phylogeny. From this point of view, the studies on many genera have been included, although no attempt has been made to refer to all such papers. It will be seen that each genus has its problems, and some peculiar situations are discovered which require further analysis. In *Narcissus* a hexaploid species has three pairs of nucleoli of very different sizes. They arise at different time intervals, the smallest pair appearing last, which is further evidence of competition between organizers for nucleolar material.

In *Poinciana regia* (Leguminosae) there are seven nucleoli in the cells, indicating that the chromosome number $(2n = 28)$ is heptaploid and the basic number four. There is further evidence from *Cicer, Clitoria, Sesbania* and other genera that four is the basic number in this large family. In the history of cultivated wheat there is evidence that *Triticum* and *Aegilops* species have both played a part. The further study of genomes with their sats. and nucleoli, combined with the identification of genomes by the pairing of chromosomes in hybrids at meiosis, should make it possible to trace out the entire history. Rice is shown to be a sec-

ondary tetraploid, while several species of *Oryza* with 48 chromosomes have eight nucleoli and are clearly secondary octoploids.

In the genus *Allium* and occasionally in other genera tandem sats. arise through interchange between two members of a sat. pair. *A. amplectens* ($2n = 14$) has three pairs of sats. and six nucleoli, while *A. Bidwelliae* ($2n = 28$) has five pairs of sats. and ten nucleoli. Further investigations are required to explain these peculiar conditions as well as the *Oenothera* species with five nucleoli.

It was formerly suggested that the nucleolar chromosomes in rice are terminally attached in prophase, but minute satellites were subsequently observed. The very large chromosomes of *Trillium* also have minute sats., so that their attachment is not strictly terminal. There are probably cases, however, in which the sat. and thread are lost but the nucleolar organizer remains, so that the attachment is truly terminal. In such genera as *Trillium* and *Haworthia* the sats. are probably being gradually reduced below the limits of visibility. They may be regarded as vestigial organs of the cell. In various other genera it appears that a pair of sats. can be suddenly lost through a mutation.

In *Brassica*, where amphidiploidy has taken place repeatedly between species with different chromosome numbers, the nucleoli and sats. are of great value in tracing the parents of the various crosses. In the related genus *Crambe*, 15 is the basic number and species are known to be $2n$, $4n$, $6n$, $12n$ on this basis, but their nucleoli have not yet been studied. The presence of eight nucleoli in species of *Paeonia* with only $2n = 10$ chromosomes requires a special explanation if the condition is correctly reported.

In pollen mother cells the zygotene pairing of the nucleolar chromosome threads drags together their nucleoli, but they are frequently prevented from fusing by the paired threads which lie between them. This was formerly sometimes regarded as nucleolar budding, but true budding also occurs. According to the best observations, the budding in pollen mother cells of rice and *Antirrhinum* is always at the point of attachment of the nucleolus to the chromosome. The bud grows until both nucleoli are the same size. As the bud is paler staining and disappears earlier then the parent nucleolus, this process probably separates two different substances. Budding also occurs in the nucleoli of some animal eggs in connection with the process of yolk formation.

Special studies of the nucleoli in the salivary glands of insects show that in *Chironomus* there are two pairs of very large nucleoli although there are only four pairs of chromosomes.   The two pairs of nucleoli are of different size and structure, indicating that they are also functionally different.   The banded structure of the chromosomes is modified at the region where the nucleoli arise.   In addition, Neben-nucleoli or small droplets are produced from the chromosomes at certain bands.   Thus the nucleoli are simply the most conspicuous of the bodies which are produced by the chromosomes at particular loci through interactions with the surrounding medium.   In *Sciara* no nucleoli can be seen, but "bulbs" and "puffs" on the banded salivary chromosomes may contain nucleolar material.   This condition of *Sciara* appears to be unique.

In groups of organisms as far apart as insects and liverworts the sex chromosomes appear to be also generally the nucleolus-producing chromosomes.   The nature of this relationship is not at present clear.

Finally, we may conclude that the number of nucleoli in the cells of a species is probably as important phylogenetically as the number of chromosomes.   Future reports of chromosome numbers should include at least a determination of the number of chromosomes with sats. or secondary constrictions and the number and sizes of the nucleoli in somatic telophase.   In animal cytology it is important that more attention be paid to the numbers of nucleoli in somatic telophase and their relation to sat-chromosomes and the chromosome numbers in different species.

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THE BOTANICAL REVIEW

VITAMIN DEFICIENCIES OF THE FILAMENTOUS FUNGI

WILLIAM J. ROBBINS AND VIRGENE KAVANAGH

New York Botanical Garden and Department of Botany, Columbia University

It is now well recognized that the growth of many fungi is determined by the presence or absence in the culture medium of vitamins, their components or derivatives. These fungi grow poorly or not at all in a medium limited to minerals, sugar, water and a favorable source of nitrogen, but develop satisfactorily in the same medium supplemented by extracts of various natural products, or by minute amounts of one or more vitamins in chemically pure form. Information on this aspect of the physiology of fungi has accumulated rapidly. The subject is of fundamental importance not only to the culture of specific fungi, but also to our knowledge of vitamins (so important for animals including man), and to a clear understanding of the basic factors concerned in the development of all organisms. Because of the ease and convenience with which they can be cultivated, fungi may give us information on vitamins more quickly than can be obtained with experimental animals.

In the succeeding pages the authors have summarized the literature on the relation of vitamins to the growth of specific fungi. We have included in this survey those fungi which are reported to grow poorly or not at all in solutions limited to minerals, sugar and a source of nitrogen. The name of each fungus[1] (as stated by the author) is given with the general composition of the basal medium used and the effect on development of the fungus of the addition of natural extracts or pure vitamins. We have not limited ourselves to those fungi on which the effect of pure vitamins has been studied, but have included also those on which extracts of natural products have been reported to have a beneficial effect, even though the cause of the benefit has not been determined. The bacteria and yeasts,

[1] We have omitted authorities for specific names even when the author included them.

except for a few torulae, have been omitted from this survey. We have listed also a few of the fungi which develop satisfactorily in a simple basal medium without the addition of vitamins or extracts from natural products.

A brief discussion of the relation of vitamins to the development of fungi will make the survey more intelligible to the average reader.

Historical. Conclusive evidence of the importance of vitamins for fungi depended upon isolation of vitamins in chemically pure form. Crystalline thiamine (vitamin B_1) was isolated by Jansen and Donath in 1926. It did not, however, become generally available until 1934. Schopfer, who had been investigating the reason for good growth of the bread mold, *Phycomyces,* on media prepared with some samples of maltose, and its failure to grow on those made with others, found the presence of thiamine in the favorable samples to be the cause of this difference. He demonstrated (106) in 1934 that addition of crystalline thiamine to a solution of pure sugar, minerals and asparagine made growth of *Phycomyces* possible in such a medium.[2] This was the first completely satisfactory demonstration of the importance of a vitamin for the growth of a fungus. Earlier work by Wildiers (139), Bachman (4), Linossier (50), Willaman (140), R. J. Williams (141), Lepeschkin (46), McCormick (54) and others was not entirely convincing because the preparations they used as supplements were not pure chemical substances.

General concepts. Although much remains to be discovered on the relation of vitamins to the growth of fungi, the following may be taken as a general statement of the present stage of our knowledge:

In the development of any fungus a considerable number of vitamins is essential. Many fungi, for example, *Aspergillus niger,* are able to make from sugar, minerals and a nitrogen source all the vitamins which they require in amounts adequate for normal and perhaps maximum development. Others suffer from one or more vitamin deficiencies;[3] that is, they do not develop satisfactorily in

[2] Burgeff had previously tested the effect of crystalline thiamine on *Phycomyces* with positive results but his publication appeared somewhat later than that of Schopfer (14, 104).

[3] Two classes of vitamin deficiencies should be distinguished. One is physiological in character; the organism is unable to synthesize the vitamin. The other is a lack of a vitamin in the food. An organism which suffers from a

a medium which lacks vitamins. Some fungi have a *complete* deficiency for one or more vitamins. They are unable to synthesize any of the vitamin (or vitamins) in question, and in its absence do not grow. This is true of *Phycomyces* for thiamine. Others suffer from *partial* deficiencies; that is, they grow slowly in the absence of the particular vitamin, but more rapidly if it is present in the medium. Apparently they are able to make some of the vitamin, but not enough for maximum growth. Both complete and partial deficiencies may be *single* (for one vitamin) or *multiple* (for more than one vitamin). The deficiency may be *absolute,* or it may be *conditioned.* By an absolute deficiency we mean that no known environmental conditions enable the organism to synthesize the vitamin from the simple foods and nutrients in a basal solution. This appears to be true of *Phycomyces* in its relation to thiamine. *Pythium butleri,* on the other hand, suffers from a thiamine deficiency in a concentrated mineral solution which is relieved by diluting the solution (90, 99). Its deficiency is conditioned by the medium in which it is grown.

The synthetic ability of a fungus for a particular vitamin may be *complete, incomplete* or *none;* that is, some fungi are able to construct the vitamin from simple foods and nutrients; others are capable of making the vitamin if supplied one or all of its intermediates; and still others are incapable of constructing any portion of the vitamin. For example, *Aspergillus niger* has complete synthetic power for thiamine; it can make this substance if supplied with sugar and minerals including nitrates (122). On the other hand, *Phytophthora cinnamomi* must be supplied with thiamine as such (78). It apparently lacks the ability to synthesize any portion of the thiamine molecule, resembling animals in this respect. Between the two extremes of no synthetic power and complete synthetic ability, there exist many types of incomplete synthetic power. For example, *Mucor ramannianus* can make the pyrimidine[4] half of the thiamine molecule but not the thiazole portion (62); *Sclerotium rolfsii* can make the thiazole but not the pyrimidine part

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physiological deficiency manifests such a condition only when its food is deficient also.

[4] When the terms pyrimidine and thiazole are used in this paper without other qualification they refer to 2-methyl-5-bromo-methyl-6-aminopyrimidine and 4-methyl-5-β-hydroxyethyl thiazole, respectively. These are the two intermediates of thiamine.

(87) ; *Phycomyces* can combine the two intermediates into the thiamine molecule but is incapable of making either (86, 111, 127).

Function of the vitamins. Some vitamins are known to be precursors of coenzymes (133). A deficiency of one of these vitamins interferes with the activity of an enzyme system and prevents normal metabolic changes accomplished through the agency of that system. For example, cocarboxylase is the pyrophosphate of thiamine. The enzyme, carboxylase, catalyzes the decarboxylation of pyruvic acid, one of the intermediates in the metabolism of glucose; but carboxylase is effective only in the presence of its coenzyme, cocarboxylase. When thiamine is deficient and cocarboxylase is not formed, carboxylase does not function, and the normal utilization of sugar does not occur.

Specificity. Their function as coenzymes probably explains the high degree of specificity shown by vitamins. Investigations of analogs of thiamine and its intermediates, particularly with *Phycomyces,* illustrate this specificity. As may be noted later in this paper, even a small change in the molecular structure of the pyrimidine or the thiazole portion of the thiamine molecule reduces its effectiveness, or eliminates it entirely. There are, however, some differences between organisms in their response to certain analogs. Compare the response of *Phytophthora fagopyri* and *Parasitella simplex* to 2-ethyl-6-amino-5-aminomethyl pyrimidine, as reported by Schopfer and Blumer (120). Perhaps certain fungi are able to transform some of the closely related compounds into thiamine and others are not.

Vitamins and growth substances. The substances concerned in this review include more than the vitamins as such, and may be called *growth substances.* A growth substance, in the specialized sense in which the term is used in this paper, refers to a definite organic compound, minute amounts of which when added to the medium have a materially favorable effect upon development of a fungus. Compounds which serve as growth substances are produced by some fungi, as was pointed out earlier, and function in the same way for these organisms as they do for those which must be furnished growth substances in the medium. Many growth substances are specific and indispensable (93*a*). Some are vitamins, others are vitamin derivatives or components, and still others cannot be classified properly with our present knowledge. We prefer

for the present the more general term growth substances to the various specialized names which have been suggested.[5]

Funk (27) did not limit the term vitamin(e) to substances of significance for animals, but considered that vitamins might be important for plants also. In the authorized English translation of the second German edition of his book on vitamins he approved Willaman's definition (140), which is as follows:

"Vitamins constitute a class of substances the individuals of which are necessary for the normal metabolism of certain living organisms but which do not contribute to the mineral, nitrogen or energy factors of the nutrition of these organisms."

While this definition might be interpreted to cover all the substances included in this review, nevertheless, the term vitamin has come by usage to have certain connotations which make its extension awkward. For example, pyrimidine is a growth substance for *Sclerotium rolfsii* and thiazole for *Mucor ramannianus,* yet we hesitate to call either of these compounds a vitamin. The vitamin concerned is thiamine. Pyrimidine functions as a growth substance for *Sclerotium rolfsii* because thiamine is essential for development of the fungus, and the latter can construct this necessary material if furnished pyrimidine, but not otherwise.

Of the dozen or more chemically pure vitamins, and similar substances now available, relatively few have been found to be growth substances for fungi. Deficiencies for thiamine or for its components and derivatives (pyrimidine, thiazole, cocarboxylase, thiochrome) are relatively common; biotin deficiencies are numerous; pyridoxine deficiencies are infrequent; *i*-inositol is a growth substance for some fungi and oleic acid for one (7). Deficiencies for riboflavin, nicotinic acid, the auxins,[6] pantothenic acid, pimelic acid, para-aminobenzoic acid, vitamin K, ascorbic acid, and the fat-soluble vitamins have not been reported.[7] Investigation of the growth substance relations of fungi is still in its beginning, and further research will doubtless add to the list of organisms which have deficiencies and to the list of compounds which function as growth substances.

[5] Numerous other terms have been proposed for these substances (auximones, auxithals, nutrilites, ergones) but none has been generally accepted.

[6] Except possibly for *Pyronema confluens.*

[7] Beadle and Tatum (6) have recently reported mutant strains of *Neurospora* produced by treatment of spores with x-rays which are deficient for pyridoxine, and some deficient for para-aminobenzoic acid.

The amounts of the growth substances effective in promoting growth are minute as compared with the sugar or nitrogen used in metabolism. The microgram (μg) or the millimicromole (mμ mole) is the unit of measurement rather than the gram or mole. *Phycomyces* responds to 0.01 mμ mole of thiamine, and maximum effects are obtained with 2 or 3 mμ moles in 25 ml. of medium. Positive effects with 0.0001 μg of biotin on the growth of *Ashbya gossypii* have been observed (40). On the other hand, *i*-inositol is used in quantities measured in milligrams. In spite of the small quantities involved, it should be emphasized that the vitamins or similar substances are used up by the organisms.

Some fungi have been found useful for vitamin bioassays. Those so employed have complete deficiencies for a given vitamin. They respond to minute amounts by growth which is proportional to the quantity of the vitamin present, and the response is quite specific (see *Ashbya gossypii, Lophodermium pinastri, Mucor ramannianus, Phycomyces blakesleeanus, Phytophthora cinnamomi* and *P. erythroseptica*).

The methods of investigation in this field are exacting. Because of the minute amounts of growth substances which are effective and because of their wide distribution in products of natural origin, special attention must be given to cleanliness of glassware and other utensils and to purity of the chemicals. Effective quantities of one or more vitamins may be present in the carbohydrates used, in agar, in gelatin, in cotton, in cheesecloth, in asparagine or in any other product of natural origin (19, 94, 95). Growth inhibitors which may be present in products of natural origin may interfere with the effectiveness of specific vitamins. Some growth inhibitors and some vitamins have been found to be mutually antagonistic, *i.e.,* the injury produced by the inhibitor is reduced by additions of the vitamin, or the benefit from the vitamin is decreased in the presence of the inhibitor.[8] The growth inhibitor may reduce the physiological availability of the vitamin by combining with it (uncooked egg white and biotin) (22); or the inhibitor may compete with the vitamin for the specific enzyme protein (sulfanilamide and para-aminobenzoic acid) (24, 145). Sterilization by heat may inactivate a growth

[8] This relation has been found to be important in chemotherapy and may prove significant in the control of specific fungi. The basis for such treatments is to antagonize those vitamins for which the organism shows partial or complete deficiencies.

substance though many are thermostable; sterilization by filtration may result in adsorption of some or all of it.   In experiments involving growth substances the basal medium should be adequate in all other respects, and should approach as nearly as possible that most suitable for the organism in question.   The trace elements or minor mineral elements require especial attention (131), and the possibility must be considered that the supplements which are added correct the toxicity of the medium rather than a vitamin deficiency (2).   Environmental conditions may play a rôle (see conditioned deficiencies).   The rate of growth probably affects the demand for a particular growth substance; partial deficiencies may appear during rapid growth which are not evident when the same organism grows slowly.   Since a reserve of growth substance in the organism being studied may compensate for a deficiency in the medium, successive passages in a medium free of growth substances are advisable.   Differences have been observed in the vitamin deficiencies of species of the same genus or of strains or races of the same species (119).   Mutations resulting in a change in vitamin deficiencies have been described (6, 94).

Many of the records summarized here are concerned with growth only.   It is important to know that by addition of one or more vitamins a fungus can be made to grow in a medium in which it does not otherwise develop.   However, a record of growth does not mean that the medium is adequate for maximum growth, or satisfactory for gametic or agametic reproduction.

It is impossible in the space available here to discuss adequately the relation of fungi to vitamins.   We hope, however, that this brief summary and the records for individual organisms which follow will be useful to those interested in these matters.

Absidia coerulea (+ and −) grew in mineral-dextrose solution containing asparagine.   Addition of wheat-germ extract improved growth; thiamine or lactoflavin (impure[9]) was ineffective (104, 108).   Addition of heteroauxin was ineffective (43).

Absidia glauca (+ and −) grew in mineral-dextrose solution containing asparagine.   Addition of wheat-germ extract improved growth; thiamine or lactoflavin (impure) was ineffective (108).   Zygospore production was scanty on mineral-dextrose medium

[9] A preparation of lactoflavin used by Schopfer and by Bünning appears to have been impure.

containing KNO_3 and 1.5 per cent agar. It was greatly increased by addition of lentil extract or its inositol-free fraction (29). The fungus grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Absidia orchidis (+) grew in a mineral-dextrose solution containing asparagine. Addition of wheat-germ extract improved growth; thiamine or lactoflavin (impure) was ineffective (108).

Absidia ramosa did not grow in a mineral-dextrose solution containing asparagine. Addition of thiamine permitted growth, but addition of wheat-germ extract was much more effective (104, 108). A mixture of thiazole and pyrimidine was as effective as thiamine. Pyrimidine added singly was partially effective and thiazole had no effect (113, 115). Ethyl thiamine was as effective as methyl thiamine. Eight pyrimidine analogs were ineffective (see *Polyporus adustus*). Thiochrome was ineffective (120).

Absidia repens (+ and −) grew in mineral-dextrose solution containing asparagine. Wheat-germ extract improved growth but less than for *A. glauca* (108).

Absidia spinosa. Zygospore production was scanty on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar but was greatly increased by addition of lentil extract or its inositol-free fraction (29). Thiamine had no effect on growth or zygospore production (14).

Acanthorhynchus vaccinii grew fairly well on addition of thiamine, grew better on addition of a biotin concentrate (45).

Achlya conspicua grew on mineral-dextrose medium containing *l*-cystine and two per cent agar. Substitution of several other amino acids and thiamine for *l*-cystine was ineffective (44).

Acladium castellanii grew poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect but a concentrate of rice polishings permitted good, though slow, growth (75).

Actinomyces albus grew in a mineral-dextrose medium containing nitrate. It synthesized thiamine (51).

Actinomyces eppinger did not grow or grew very poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine or of a concentrate of rice polishings was ineffective. The mold grew slowly but fairly well

on Sabouraud's medium containing sugar and a concentrate of rice polishings (75).

Agaricus campestris grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Allomyces javanicus did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture or thiamine with amino acids was ineffective. Growth occurred when yeast extract was added to the agar medium (44). Addition of heteroauxin was ineffective (43). Almost no growth was obtained in mineral-dextrose solution containing KNO_3. Addition of a bios preparation from yeast markedly increased growth (97). *A. javanicus* was tentatively listed as a biotin-deficient organism (45).

Aphanomyces camptostylus grew on mineral-dextrose medium containing *l*-cystine and two per cent agar. Several other amino acids and thiamine did not replace *l*-cystine (44).

Aphanomyces phycophilus did not grow on cornmeal agar, grew somewhat on yeast-cornmeal agar, grew vigorously on maltose-peptone agar, but addition of thiamine to the latter medium increased growth (137).

Armillaria mellea grew in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar but did not fruit. Addition of lentil extract improved growth but did not permit fruiting (29).

Ascobolus denudatus grew in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar but produced apothecia freely only on addition of lentil extract. Inositol-free lentil extract was ineffective (29).

Ascobolus furfuraceus formed perithecia only in cultures containing bacteria (58, 59).

Ascobolus leveillei grew in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar but produced apothecia freely only on addition of lentil extract. Inositol-free lentil extract was ineffective (29).

Ascobolus viridulus did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture or thiamine with amino acids was ineffective. Growth occurred when yeast extract was added to the agar medium (44). No perithecia developed in light on

mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract induced perithecia, but inositol-free lentil extract was ineffective (29).

Ascochyta pisi grew in mineral-sucrose solution containing KNO_3. Addition of a bios preparation from yeast increased growth (97).

Ashbya (Nematospora) gossypii did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of thiamine or *i*-inositol singly or together had no effect. Addition of biotin methyl-ester improved growth. A mixture of biotin and thiamine was no better than biotin alone. A mixture of biotin and *i*-inositol was considerably better than biotin alone, but a mixture of the three growth substances gave the best results (25, 31, 40, 96). Addition of riboflavin to mineral-dextrose medium containing NH_4NO_3 and agar was ineffective (44).

Aspergillus aureus grew in mineral-sucrose solution containing NH_4NO_3. Addition of beer-yeast improved growth (74).

Aspergillus awamori grew in mineral-sucrose solution containing NH_4NO_3. Addition of beer-yeast improved growth (74).

Aspergillus chevalieri. A lentil extract greatly increased conidial production and slightly increased production of perithecia with media containing high concentrations of dextrose (29).

Aspergillus clavatus grew in mineral-sucrose solution. Maltose was better than cane sugar; dextrose, lactose, glycerine or mannitol was poorer. Growth in a ten per cent malt extract was considerably better than in mineral-sugar solutions (37).

Aspergillus flavus grew in mineral-sugar solution containing NH_4NO_3 (72).

Aspergillus fumigatus grew well in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect, but a concentrate of rice polishings improved early growth (75).

Aspergillus giganteus grew in mineral-sucrose solution. Maltose was better than cane sugar; dextrose, lactose, glycerine or mannitol was poorer. Growth in a ten per cent malt extract was considerably better than in mineral-sugar solutions. Addition of plant extracts to mineral-sucrose solutions markedly improved growth. See *Aspergillus luchuensis* (37).

Aspergillus glaucus grew in mineral-sucrose solution. Maltose was better than sucrose; dextrose, lactose, glycerine or mannitol was

poorer. Growth in ten per cent malt extract was considerably better than in mineral-sugar solutions (37). *Aspergillus glaucus* and the large group of closely related species grew well and formed perithecia on mineral-sucrose medium containing agar and 20 per cent sucrose (134).

Aspergillus gymnosardae grew in mineral-sucrose solution containing NH_4NO_3 (74).

Aspergillus luchuensis grew in mineral-sugar solutions. Maltose was better than sucrose; dextrose, lactose, glycerine or mannitol was poorer. Growth in a ten per cent malt extract was considerably better than in mineral-sugar solutions. Addition of thiamine and vitamin B_2 to the mineral-sucrose solution was ineffective. Addition of aqueous extracts of fir, pear, or mustard leaves, fir or pear mistletoe or yeast increased the five-day yield up to ninety times that obtained in mineral-sucrose solution. In some instances benefit was observed on addition of 0.007 mg. dry matter per culture. Addition of aqueous extracts of spores of *A. luchuensis* was beneficial (37).

Aspergillus niger grew in mineral-dextrose solution containing KNO_3. Addition of a bios preparation increased growth. Addition of pure auxin *a* had no effect (97). *A. niger* grew at 33° C. in mineral-sugar solution containing ammonium tartrate. Additions of the liquid from *Rhizopus suinus* grown on mineral-dextrose solution containing ammonium tartrate increased the growth of *Aspergillus* up to nine times in the first three to five days (64). Ethyl ether or ninety per cent alcohol extracts of the *Rhizopus* medium were ineffective. Autoclaving or oxidation with perhydrol did not influence the activity of extracts (67). Pentoses or hexoses heated in solutions containing organic acids or their salts produced a solution which increased growth of *Aspergillus* in mineral-sugar medium containing ammonium tartrate and ten mineral supplements (66, 68). Glyoxylic acid, a mixture of glycolic acid and pyruvic acid or a mixture of ascorbic acid, pyruvic acid and glycolic acid increased growth when added to the basal solution (69). Liquid from a *Rhizopus suinus* culture lost its beneficial action after being shaken with *Aspergillus* mycelium but was unaffected by treatment with yeast (70). The culture medium from cultures of *A. niger, Rhizopus suinus* or *Penicillium roquefortii,* extracts of yeast or *Boletus edulis,* beer

wort, an autoclaved mixture of dextrose and ammonium tartrate
and a mixture of glycolic and pyruvic acid markedly increased
three day growth at 32° C. of *A. niger*. The various materials
were added to a mineral-sucrose solution containing ammonium
sulfate and ten mineral supplements (65). Addition of β-alanine
to a mineral-sugar solution containing asparagine, glutamic acid
and thiamine had no effect on early growth (71). Addition of
thiamine to mineral-dextrose solution containing ammonium tar-
trate had no effect, but malt or yeast extract improved growth
(25). Addition of the ether-insoluble fraction of *Rhizopus
suinus* to mineral-dextrose solution caused marked acceleration
of growth and formation of spores. Addition of thiamine was
ineffective, but addition of 0.5 mg. of lactoflavin (probably im-
pure) per flask increased growth (13). Addition of aqueous
extracts of pear or mustard leaves, of yeast, or of fir or pear
mistletoe to mineral-sucrose solution containing zinc, manganese
and boron markedly increased early growth. The results were
similar to those obtained with *A. luchuensis* (37). *A. niger* was
grown for six successive passages in mineral-sucrose solution with
no diminution in dry weight (86).

Aspergillus ochraceus grew in mineral-sucrose solution containing
NH_4NO_3. Addition of beer yeast improved growth (74).

Aspergillus oniki grew in mineral-sucrose solution containing
NH_4NO_3 (74).

Aspergillus oryzae grew in mineral-sucrose solution containing
NH_4NO_3 (74).

Aspergillus ostianus grew in mineral-sucrose solution. Maltose
was better than sucrose; dextrose, lactose, glycerine or mannitol
was poorer. Growth in malt extract was considerably better
than in mineral-sugar solutions (37).

Aspergillus repens. Lentil extract greatly increased conidial pro-
duction and slightly increased production of perithecia with media
containing high concentrations of dextrose (29).

Aspergillus soya grew in mineral-sucrose solution containing
NH_4NO_3. Addition of beer yeast improved growth (74).

Basidiobolus ranarum did not grow on mineral-dextrose medium
containing NH_4NO_3 and two per cent agar but grew upon addi-
tion of an amino-acid mixture to the agar medium (44). Addi-
tion of heteroauxin was ineffective (43). The fungus grew in

mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87). *Basidiobolus* produced no zygospores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of a lentil extract induced zygospores but the inositol-free fraction was ineffective (29).

Blakeslea trispora (*Choanephora persicaria*) did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar or in liquid medium. Addition of yeast extract or thiamine to agar medium or of thiamine to liquid medium permitted growth. Thiamine intermediates or pyrimidine alone took the place of thiamine; thiazole alone was ineffective (44). Addition of heteroauxin was ineffective (43). The fungus did not grow in mineral-dextrose solution containing asparagine but grew well on addition of thiamine. Wheat-germ extract was no better than thiamine (108).

Boletus elegans grew little in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine increased dry weight seven times, addition of yeast extract ten times (56).

Boletus granulatus grew in the first passage in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine increased growth. Biotin or inositol, singly or together, were ineffective even with thiamine (57).

Boletus luteus did not grow in mineral-dextrose solution containing ammonium tartrate unless thiamine was present (57).

Boletus piperatus grew little in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine increased dry weight five times. Biotin methyl-ester reduced growth in presence of thiamine (57).

Boletus variegatus did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth (57).

Boletus viscidus did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine improved growth but was not adequate. Biotin methyl-ester and inositol singly or together were ineffective even in the presence of thiamine (57).

Bombardia lutea. Germination of ascospores was not improved by addition of β-indole-acetic acid to a maltose-agar medium (144).

Botrytis allii grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$ (147).

Botrytis cinerea.   Spores germinated in plant extracts only (12).

Cadophora fastigiata grew poorly in nutrient media lacking growth substances.   Its growth was markedly increased by growth substances of the bios group (77).

Catenularia sp. grew in mineral-dextrose solution containing asparagine.   Addition of thiamine or of its intermediates had no effect (121).

Cenococcum graniforme did not grow in mineral-dextrose solution containing ammonium tartrate.   Addition of thiamine permitted growth.   Addition of biotin methyl-ester and inositol singly or together to mineral-dextrose solution was less effective than thiamine.   Biotin and inositol did not improve effectiveness of thiamine (57).

Cephalosporium recifei grew on mineral-sugar medium containing agar (1).

Cephalosporium sp. grew in mineral-sucrose solution containing $NaNO_3$ and $(NH_4)_2HPO_4$.   Addition of amino acids improved growth during the first four days but yeast extract was more effective (142).

Ceratostomella adiposum.   Pycnidia were sparse on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar.   Addition of a lentil extract resulted in production of numerous pycnidia.   The inositol-free fraction of lentil extract was ineffective (29).

Ceratostomella fimbriata grew very little in mineral-dextrose medium containing asparagine with or without 1.5 per cent purified agar.   Addition of biotin and pyridoxine, singly or together, did not improve growth.   Addition of thiamine permitted fair growth; it was improved by further addition of biotin or pyridoxine, and the three vitamins permitted best growth.   Addition of 8 other vitamins and 21 amino acids, or of malt extract or peptone, did not increase the growth obtained with these three vitamins (95).

Ceratostomella ips.   One of two strains did not grow in mineral-dextrose medium containing asparagine with or without 1.5 per cent purified agar.   Addition of thiamine or pyridoxine, singly or together, did not permit growth.   Addition of biotin permitted good growth, but it was improved by the further addition of either thiamine or pyridoxine, and the three vitamins together were still more effective.   Addition of eight other vitamins and 21 amino

acids to this solution did not improve growth. Malt extract tripled the growth obtained with the vitamins, and an aqueous extract of cotton fibers was still more beneficial. The second strain did not grow in mineral-dextrose media containing asparagine with or without 1.5 per cent agar. Addition of thiamine, of pyridoxine, or of thiamine and pyridoxine had no effect. Addition of biotin, of biotin and thiamine, or of biotin and pyridoxine permitted some growth. Addition of biotin, pyridoxine, and thiamine produced good growth. Addition of malt extract, peptone, or of 8 other vitamins and 21 amino acids did not increase the growth obtained with the three vitamins (95).

Ceratostomella montium did not grow in mineral-dextrose media containing asparagine with or without 1.5 per cent purified agar. Addition of thiamine, of pyridoxine, or of thiamine and pyridoxine had no effect. Addition of biotin, of biotin and thiamine, or of biotin and pyridoxine permitted slight growth. Addition of thiamine, pyridoxine and biotin produced good growth. The addition of 8 other vitamins and 21 amino acids, or of neopeptone had little effect. Malt extract improved growth somewhat (95).

Ceratostomella multiannulata did not grow on a mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture, or thiamine with amino acids was ineffective. Growth occurred when yeast extract was added to the agar medium (44). The fungus was tentatively listed as a biotin-deficient organism (45).

Ceratostomella piceaperda grew slowly on mineral-dextrose medium containing asparagine and 1.5 per cent agar. Addition of thiamine had little effect; addition of biotin or of pyridoxine increased growth but addition of the three vitamins gave most growth. The fungus grew very little in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect; but biotin or pyridoxine improved growth, pyridoxine more than biotin. Thiamine and biotin, or pyridoxine and biotin gave growth about equal to pyridoxine alone; addition of thiamine to pyridoxine improved growth. The three vitamins were considerably more effective than any combination of two. Addition of calcium pantothenate, nicotinamide, para-aminobenzoic acid and *i*-inositol to the solution containing biotin, thiamine, and pyridoxine increased growth and the addition of lactoflavin, ascorbic

acid, pimelic acid and glutamine improved growth still more
(95).

Ceratostomella pini (2 strains) did not grow in mineral-dextrose
media containing asparagine with or without 1.5 per cent puri-
fied agar. Addition of biotin, of pyridoxine, of thiamine, of
biotin and pyridoxine, or of pyridoxine and thiamine had little or
no effect. Addition of biotin and thiamine or of the three vita-
mins permitted good growth (95).

Ceratostomella pseudotsugae grew slowly on mineral-dextrose media
containing asparagine and 1.5 per cent purified agar. Addition
of biotin did not increase growth. Pyridoxine increased the rate
of growth on agar somewhat and thiamine was distinctly bene-
ficial; biotin and pyridoxine were more effective than pyridoxine
alone. Pyridoxine and thiamine, biotin and thiamine, or all three
vitamins permitted good growth. In mineral-dextrose solution
containing asparagine, the fungus made small growth. Addition
of biotin alone had no effect; pyridoxine improved growth in the
second passage; and thiamine permitted good growth. Combina-
tions of the two or three vitamins were not consistently better
than thiamine alone. Eleven vitamins and 21 amino acids, or
pyridoxine, thiamine and neopeptone, or malt extract were some-
what more effective than the three vitamins (95).

Ceratostomella ulmi grew better with addition of a bios preparation
from yeast (41). *C. ulmi* grew slightly or not at all in mineral-
dextrose medium containing asparagine with or without 1.5 per
cent purified agar. Addition of thiamine and biotin, singly or
together, or of nicotinamide, calcium pantothenate, *i*-inositol,
or para-aminobenzoic acid did not improve growth. Addition of
pyridoxine permitted good growth, which was not improved by
any of the other pure substances tried. Addition of peptone or
malt extract produced from two to four times the growth obtained
with pyridoxine (95).

Ceratostomella from London plane tree grew very little in mineral-
dextrose solution containing asparagine or in the same medium
plus 1.5 per cent purified agar. Addition of biotin and pyri-
doxine, singly or together, did not improve growth in either
medium. Addition of thiamine permitted growth, better growth
was obtained with thiamine and pyridoxine or thiamine and
biotin. Best growth was obtained with all three vitamins. Addi-

tion of 8 other vitamins and 21 amino acids did not improve growth (95).

Cercospora apii grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$ (147).

Cercospora beticola grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$ (147).

Cercosporella herpotrichoides grew very little in mineral-dextrose solution containing KNO_3. Addition of a bios preparation from yeast caused a marked increase (97).

Chaetocladium brefeldii did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of yeast extract gave good growth; thiamine, fair growth; thiamine and amino acids, good growth. *Chaetocladium* did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of thiamine was ineffective, but addition of thiamine and amino acids gave good growth (44). Addition of heteroauxin was ineffective (43). The fungus did not grow in mineral-dextrose solution containing asparagine, and grew poorly on addition of thiamine or wheat germ extract (108).

Chaetocladium macrosporum grew poorly on mineral-sugar media containing asparagine and agar. Addition of thiamine permitted good growth (14).

Chaetomium bostrychodes grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Chaetomium cochlioides grew and fruited freely on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract did not improve fruiting (29).

Chaetomium elatum grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Chaetostylum fresenii showed no response to thiamine (14).

Choanephora cucurbitarum did not grow in mineral-dextrose solution containing asparagine but grew well on addition of thiamine. Wheat germ extract was no better than thiamine (108).

Circinella aspera grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Circinella spinosa grew in mineral-dextrose solution containing NH_4NO_3. Addition of heteroauxin was ineffective (43).

Cladosporium herbarum grew in mineral-cane sugar solution. Addition of plant extracts markedly improved growth. See *Aspergillus luchuensis* (37).

Clitopilus prunulus grew little in mineral-dextrose solution. Addition of thiamine increased dry weight eighteen times. Yeast extract was less favorable than thiamine (56).

Colletotrichum circinans grew on mineral-sugar medium containing 1.7 per cent agar and a trace of ammonium tartrate but addition of extracts of *Rhizopus suinus* increased growth as much as two times (146).

Colletotrichum gloeosporioides. Lactoflavin, thiamine, nicotinic acid, ascorbic acid, carotene, vitamin E, malic acid, inositol, pimelic acid or amino acids induced germination of spores from corn-meal agar cultures. Lactoflavin only was effective for spores from oat-meal agar cultures (18).

Colletotrichum lindemuthianum grew in mineral-sucrose solution containing KNO_3. Addition of a bios preparation from yeast had little effect (97).

Collybia tuberosa did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Good growth was obtained on addition of thiamine or yeast extract to agar medium. *Collybia* did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of thiamine to the liquid medium produced little or no growth, but addition of amino acids and thiamine was effective. Thiazole and pyrimidine or pyrimidine alone took the place of thiamine; thiazole alone was ineffective. No growth was obtained with yeast extract, thiamine, the intermediates or pyrimidine after they were autoclaved five hours at pH 10 (44).

Collybia velutipes grew and fruited in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract increased fruiting (29).

Conidiobolus villosus did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of yeast extract permitted good growth. Addition of heteroauxin was ineffective (43).

Coprinus lagopus did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of yeast gave fair growth. Thiamine or riboflavin was ineffective but with thiamine and amino acids good growth was obtained. The fungus grew in mineral-dextrose solution containing amino acids and

thiamine. Thiazole and pyrimidine together or pyrimidine alone substituted for thiamine. Thiazole was ineffective (44).

Coprinus radians cannot grow unless some complex organic substance is added to the nutrient solution (42).

Coprinus sterquilinus. Spores germinated only in cultures contaminated with bacteria (5).

Coprinus tergiversans was listed as a thiamine-deficient organism (45).

Corticium vagum. Addition of thiamine or yeast extract to mineral-sugar solution increased the growth of one strain eighty times (36).

Cryptococcus (Busse-Buschke) grew poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine or of a concentrate of rice polishings improved growth (75).

Cunninghamella bertholletiae grew in mineral-dextrose solution containing NH_4NO_3. Addition of heteroauxin was ineffective (43).

Cunninghamella echinulata grew in mineral-dextrose solution containing asparagine (108).

Cunninghamella elegans grew in mineral-dextrose solution containing asparagine (108).

Cunninghamella sp. grew in mineral-dextrose solution containing asparagine (87).

Cyathus striatus was listed as a thiamine-deficient organism (45).

Dacryomyces stillatus did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine gave nearly as good growth as malt or yeast extract (25).

Daedalea unicolor did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth, but greater yields were obtained with addition of yeast extract (25).

Dasyobolus immersus did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of yeast extract permitted good growth. Addition of heteroauxin was ineffective (43).

Deconica inquilina was listed as a thiamine-deficient organism (45).

Dematium chodati grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Dematium nigrum grew poorly in mineral-dextrose solution con-

taining asparagine. Addition of thiamine increased dry weight between five and six times. Pyrimidine or a mixture of pyrimidine and thiazole were as effective as thiamine. Thiazole did not replace thiamine (115). 2-methyl-5-thioformylamino-methyl-6-amino-pyrimidine and 2–5-dimethyl-6-amino-pyrimidine were partially effective in place of pyrimidine. Five other pyrimidines were ineffective (see *Polyporus adustus*) (120).

Dematium pullulans. Growth in mineral-dextrose solution containing NH_4NO_3 was accelerated by addition of aqueous extracts of camomile or mistletoe (9). The organism grew well in mineral-dextrose medium containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Dematium sp. Growth was somewhat improved by addition of thiamine, of thiazole or of a mixture of thiazole and pyrimidine to mineral-dextrose solution containing asparagine (121).

Dermatea balsamea was listed as a thiamine-deficient organism (45).

Diaporthe strumella grew in mineral-dextrose solution containing NH_4NO_3. Addition of heteroauxin was ineffective (43).

Dichranophora fulva did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine improved growth. Addition of wheat-germ extract gave an effect equal that of thiamine (108). Addition of heteroauxin was ineffective (43).

Diplodia macrospora did not grow on mineral-dextrose or mineral-sucrose medium containing NH_4NO_3, aspartic acid, glutamic acid and agar. Addition of a biotin-concentrate permitted growth, as did the substitution of a brown sugar for pure sugar (52).

Dipodascus uninucleatus did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture, or thiamine with amino acids was ineffective. Growth occurred when yeast extract was added to the agar medium (44). *Dipodascus* was tentatively listed as a biotin-deficient organism (45).

Discella carbonacea did not grow in mineral-sugar solution containing ammonium tartrate. Addition of thiamine was of little benefit. Addition of malt extract or yeast extract permitted growth (25).

Dothidella quercus grew well in the first passage in mineral-sucrose solution containing $Ca(NO_3)_2$ but less well in one containing KNO_3 (147).

Entyloma arnoseridis appeared to grow on mineral-dextrose medium containing asparagine and two per cent washed agar, and to be unaffected by addition of thiamine (121).

Eremascus fertilis grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Fomes fraxineus grew and fruited in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract improved fruiting (29).

Fomes igniarius did not grow on mineral-sugar medium containing agar and $(NH_4)_2SO_4$ or asparagine. Addition of thiamine permitted growth on the $(NH_4)_2SO_4$ medium. Addition of thiamine to peptone medium increased growth slightly but growth on potato-dextrose or malt agar was much the best (73).

Fomes pinicola did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine gave slight growth. Addition of yeast extract gave better growth than was obtained with thiamine. Addition of biotin and inositol was ineffective even in presence of thiamine (25, 40).

Fusarium avenaceum did not grow in mineral-sugar solution containing KNO_3. Addition of agar or of a filtrate from *Penicillium* cultures permitted growth (135). Two strains grew in mineral-sugar solution containing KNO_3 or asparagine. One strain did not grow in the liquid medium. Addition of agar, agar extract or biotin methyl-ester permitted growth. Mutants from this strain grew without addition of biotin (94).

Fusarium batatatis grew in the first passage in mineral-sucrose solution containing $Ca(NO_3)_2$ or KNO_3 (147).

Fusarium conglutinans var. *callistephi* grew well in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine had no effect but yeast or malt extract improved growth considerably (25).

Fusarium niveum grows well without addition of growth substances to medium (45). The fungus grew well in mineral-sucrose solution containing NH_4NO_3 or asparagine but produced no conidia. Addition of peptone, yeast bios or plant extract permitted production of spores (136).

Fusarium oxysporum grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$ (147).

Fusarium radicicola grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$ (147).

Fusarium vasinfectum grew poorly in mineral-sucrose solution containing NH_4NO_3 and produced no conidia. Growth was much better and conidia were freely produced in media containing various plant extracts (136).

Fusarium sp. (elegans section) grew well in mineral-sugar solution containing $(NH_4)_2SO_4$ but produced no conidia. Addition of melon juice improved growth and permitted spore production (136).

Geotrichoides sp. grew well on mineral-dextrose medium containing NH_4NO_3 and 1.5 per cent washed agar (77).

Gloeocystidium roseo-cremeum was listed as a thiamine-deficient organism (45).

Glomerella cingulata grew in mineral-sucrose solution. Addition of a bios preparation from yeast doubled growth (97).

Grubyella japonica grew poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect but a concentrate of rice polishings permitted good growth (75).

Grubyella ochraea grew poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect but a concentrate of rice polishings permitted growth (75).

Gymnoascus setosus grew and fruited freely on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract did not improve fruiting (29). The fungus grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Helminthosporium sativum grew in mineral-sucrose solution containing KNO_3. Addition of a bios preparation from yeast had no effect (97).

Helvella infula did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted good growth. Malt extract or yeast extract was not superior to thiamine. Addition of biotin methyl-ester and inositol was ineffective even in the presence of thiamine (25, 40).

Hydnum coralloides grew and fruited in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract increased fruiting (29).

Hydnum erinaceus did not grow on mineral-sugar medium containing agar and $(NH_4)_2SO_4$ or asparagine. Addition of thiamine permitted growth. Addition of thiamine to peptone medium increased growth fifty per cent. Growth on thiamine-peptone medium surpassed that on potato-dextrose agar but was inferior to that on malt-extract agar (73).

Hypholoma fasciculare grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine increased growth twenty times. A mixture of thiazole and pyrimidine was as effective as thiamine. Thiazole or pyrimidine used singly was ineffective (121).

Hypochnus catonii. Cladosporium herbarum induced basidiospores when uncontaminated cultures failed to fruit (17).

Hypochnus solani grew well in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine, yeast extract or malt extract had no effect (25).

Hypoxylon pruinatum did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine, of biotin methyl-ester, of inositol or of inositol and thiamine was ineffective. Addition of thiamine and biotin gave good growth which was not surpassed by further addition of inositol (25).

Indiella americana grew poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine, and sugar. Addition of thiamine or of a concentrate of rice polishings improved growth (75).

Isaria sp. did not produce coremia freely on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. On the same medium plus lentil extract coremia were freely produced (29).

Isoachlya monilifera grew on mineral-dextrose medium containing *l*-cystine and agar. The substitution of several other amino acids and thiamine for *l*-cystine was ineffective (44).

Lactarius deliciosus did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted a little growth. Yeast extract was more favorable than thiamine but the growth obtained was small (56).

Lambertella corni-maris grew on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar but no fruit bodies matured in six months without addition of lentil extract (29).

Lentinus tigrinus did not grow on mineral-dextrose medium con-

taining NH_4NO_3 and two per cent agar. Addition of yeast extract or thiamine permitted good growth. The fungus grew in mineral-dextrose solution containing thiamine and amino acids. A mixture of thiazole and pyrimidine was as effective as thiamine; pyrimidine was partially effective, and thiazole or riboflavin was ineffective (44).

Lenzites betulina was listed as a thiamine-deficient organism (45).

Lenzites sepiaria (two strains) did not grow in mineral-dextrose solution containing ammonium tartrate; addition of thiamine permitted slow but continuous growth, and addition of yeast extract, faster growth. Biotin and inositol alone or together were ineffective even with thiamine present (25, 40). The fungus was unable to utilize thiazole or pyrimidine alone or in mixture and required the thiamine molecule (45).

Lophodermium pinastri did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of thiamine, *i*-inositol or biotin methyl-ester was ineffective. Addition of a mixture of thiamine and inositol or of biotin and inositol was ineffective. Addition of biotin and thiamine permitted growth which was further improved when inositol also was added (25, 40).

Macrosporium sarcinaeforme grew well in the first passage in mineral-sucrose solution containing $Ca(NO_3)_2$ or KNO_3 (147).

Malassezia furfur? (Kambayasi) grew slowly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine or of a concentrate of rice polishings markedly improved growth (75).

Marasmius alliaceus did not grow in mineral-dextrose solution containing NH_4Cl. Addition of thiamine permitted growth. Inositol was ineffective, and biotin methyl-ester had slight effect. See *Marasmius foetidus* (49).

Marasmius androsaceus did not grow in mineral-dextrose solution containing NH_4Cl or ammonium tartrate. Addition of thiamine or inositol singly or together had no effect. Addition of biotin methyl-ester permitted some growth, but addition of thiamine and biotin together was more effective. Inositol did not increase the effectiveness of biotin. Fifty millimicrograms of biotin gave the best growth in the presence of one microgram of thiamine per flask (49).

Marasmius chordalis did not grow in a mineral-dextrose solution

containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Marasmius epiphyllus did not grow in mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Marasmius foetidus did not grow in mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth to occur. Addition of inositol was ineffective, addition of biotin methyl-ester improved growth slightly. Mixtures of thiamine and biotin were somewhat more effective than thiamine alone (49).

Marasmius fulvobulbillosus did not grow in mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Marasmius graminum did not grow in mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Marasmius perforans (two strains) did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth of one strain. Inositol and biotin methyl-ester, singly or in combination with thiamine, had little or no effect (see *M. foetidus*). For strain two, addition of thiamine or inositol alone or together had no effect, biotin alone had a slight effect, but biotin and thiamine together were quite effective. See *M. androsaceus* (49).

Marasmius peronatus did not grow in a mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Marasmius ramealis did not grow in mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Marasmius rotula did not grow in mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Marasmius scoradonius did not grow in a mineral-dextrose solution containing NH₄Cl. Addition of thiamine permitted growth. Inositol was ineffective and biotin methyl-ester had slight effect. See *M. foetidus* (49).

Melanconium betulinum did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine, of biotin methyl-ester, of inositol or of biotin and inositol had no effect. Addition of thiamine and biotin or of thiamine and inositol gave some growth but one-third or less that obtained in the presence of all three growth substances (25).

Melanospora destruens did not grow in mineral-dextrose solution containing KNO₃. Addition of inositol, or thiamine had no effect. Addition of biotin methyl-ester permitted some growth but biotin and thiamine together were much more effective. On the mineral-dextrose medium plus 1.5 per cent agar no perithecia were produced. Addition of inositol, biotin, thiamine, biotin and inositol or thiamine and inositol did not improve perithecial formation. Addition of biotin and thiamine or biotin and pyrimidine permitted formation of perithecia. Biotin and thiazole were ineffective (30, 31).

Melanospora pampeana did not produce perithecia on dextrose agar. Perithecia were produced on the same medium on which *Fusarium moniliforme* or *Basisporium gallarum* had grown. Media from younger cultures were better than those from older cultures (32).

Melanospora zamiae. Growth and perithecial formation were poor on mineral-dextrose medium containing KNO₃ and 1.5 per cent agar but good on the same medium plus lentil extract. The inositol-free fraction of the lentil extract was ineffective (29).

Merulius lachrymans did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth (25).

Microsporon fulvum grew in mineral-sucrose solution containing NaNO₃ and (NH₄)₂HPO₄. Addition of an amino acid mixture improved growth but yeast extract was more effective (142).

Monascus purpureus grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Mortierella candelabrum grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Mortierella isabellina grew in mineral-dextrose solution containing asparagine (108).

Mortierella pusilla grew in mineral-dextrose solution containing asparagine (108).

Mortierella sp. #11 grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Mucor circinelloides grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Mucor genevensis grew in mineral-dextrose solution containing NH_4NO_3. Addition of heteroauxin was ineffective (43).

Mucor griseolilacinus grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Mucor hiemalis (+ & −). Zygospores were formed more freely on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar plus lentil extract than on the same medium without lentil extract. An inositol-free fraction of the lentil extract was less effective than the lentil extract (29). The fungus grew in mineral-dextrose solution containing asparagine. Addition of thiamine and lactoflavin had no effect but wheat germ extract improved growth (104, 108).

Mucor mucedo (+ and −) grew poorly (first passage) on mineral-dextrose medium containing asparagine. Addition of thiamine or lactoflavin (impure preparation) improved growth but wheat-germ extract was much more effective (104, 108). Thiamine was ineffective (14).

Mucor mucilagineus showed no response to thiamine (14).

Mucor racemosus grew poorly in mineral-sucrose solution containing $NaNO_3$ and $(NH_4)_2HPO_4$. Addition of an amino-acid mixture improved growth somewhat but yeast extract was more effective (142).

Mucor ramannianus did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine, of thiazole, or of a mixture of pyrimidine and thiazole permitted growth; pyrimidine was ineffective (104, 108). 4-methyl-thiazole, 4,5-dimethyl-thiazole, or 2-mercapto-4-methyl-thiazole was ineffective. 3-(4′(5′)-methylimidazol-4-methyl-5-β-hydroxyethyl-thiazole was effective (62). However, 3-benzyl-4-methyl-5-β-hydroxyethyl-thiazole was ineffective (116). For the first ten days of growth thiazole was less effective than thiamine, cocarboxylase, or a mixture of

pyrimidine and thiazole. All were equally effective for twelve days growth (48).

Mucor sp. #103 and #105 grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Mucor stolonifer grew in mineral-sugar solution containing NH_4NO_3 (72).

Mucor tenuis showed no response to thiamine (14).

Myceloblastanon cutaneum grew rather poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect but a concentrate of rice polishings improved growth (75).

Mycosphaerella confusa grew in mineral-dextrose solution containing asparagine. Addition of thiamine had a slight beneficial effect (121).

Mycosphaerella grossulariae grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect. The same results were obtained on the medium containing two per cent washed agar (121). However, Schopfer & Blumer state that this organism is markedly favored by addition of thiamine or a mixture of pyrimidine and thiazole. Their recorded results are in obvious conflict with this statement. It is probable that the statement applies to *Septoria apii* and represents an error in typography.

Mycosphaerella platanifolia. Sporulation occurred in juice expressed from leaves of *Platanus racemosa* and filtered sterile. Abundant spermatia were produced on sterile filter paper wet with the juice (130).

Mycosphaerella sentina grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine or a mixture of pyrimidine and thiazole improved growth. Thiazole or pyrimidine used singly was ineffective (121).

Mycosphaerella stigmina-platini. Sporulation occurred in juice expressed from leaves of *Platanus racemosa* and filtered sterile. Abundant spermatia were produced on sterile filter paper wet with the juice (130).

Nectria coccinea grew in mineral-dextrose solution containing ammonium tartrate (first passage), but addition of thiamine doubled growth. Biotin methyl-ester and inositol singly or together had little effect even in the presence of thiamine (25, 40).

Nematospora coryli grew more vigorously on potato-extract agar than on mineral-dextrose medium containing 1.5 per cent agar and asparagine. Addition of peptone to the latter medium markedly improved growth (23).

Neurospora crassa did not grow on mineral-sucrose medium containing agar and ammonium tartrate. Addition of biotin permitted growth. A strain obtained by x-raying spores did not grow unless para-amino-benzoic acid was added to this medium (6).

Neurospora sitophila grew on corn-meal agar medium and formed sclerotia. Adrenalin increased the number of sclerotia. When + and – strains were plated together, adrenalin stimulated the formation of perithecia (60). The fungus grew slightly in mineral-dextrose solution containing asparagine. Addition of biotin methyl-ester permitted normal growth with formation of ascospores (16). One strain, obtained by x-raying spores, did not grow on mineral-sucrose medium containing agar and biotin. Addition of pyridoxine permitted good growth. A second strain, obtained by x-raying spores, did not grow on mineral-sucrose medium containing agar and biotin. Addition of thiamine or of thiazole permitted good growth (6).

Neurospora tetrasperma (homothallic strain) formed few immature perithecia on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract induced formation of mature perithecia. The inositol-free fraction of lentil extract was less effective than the lentil extract (29). This *Neurospora* grew slightly in mineral-dextrose solution containing asparagine. Addition of biotin methyl-ester permitted good growth, and perithecia were produced, but no ascospores were observed (16).

Nyctalis asterophora did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of yeast extract to the agar medium gave good growth. Addition of thiamine was ineffective, but thiamine with an amino-acid mixture was effective. The thiazole and pyrimidine together or pyrimidine alone was as effective as thiamine. Thiazole was ineffective (44).

Ophiobolus graminis. Addition of a bios preparation from yeast increased growth in mineral-dextrose solution containing KNO_3 (97). The fungus does not grow in common synthetic solutions

without the addition of certain plant or animal extracts, for example, peptone, potato extract, casein or carrot extract (76). *O. graminis* did not grow in mineral-dextrose solution containing sodium nitrate, ammonium nitrate, asparagine or glycine. Addition of inositol or thiamine was ineffective. Addition of crystalline biotin methyl-ester permitted growth which was further increased by thiamine (138).

Ophiobolus miyabeanus grew well in mineral-sucrose solution containing KNO_3 (103).

Ophiobolus oryzinus did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture, or thiamine with amino acids was ineffective. Growth occurred when yeast extract was added to the agar medium (44). *Ophiobolus* was tentatively listed as a biotin-deficient organism (45).

Ophiostoma catonianum was tentatively listed as a biotin-deficient organism (45).

Parasitella simplex did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted growth. Wheat-germ extract was no more effective than thiamine (108). *Parasitella* grew poorly on mineral-sugar media containing asparagine and agar. Addition of thiamine permitted good growth and zygote formation (14). A mixture of thiazole and pyrimidine was as effective as thiamine; pyrimidine singly was partially effective; and thiazole was ineffective (113, 115, 120). 2-methyl-5-thioformylaminomethyl-6-amino-pyrimidine and 2-ethyl-5-aminomethyl-6-amino-pyrimidine were as effective as pyrimidine. Six other pyrimidines had little or no effect (see *Polyporus adustus*). Thiochrome was effective but less than thiamine (120).

Penicillium camemberti grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (121).

Penicillium crevicaule grew in mineral-sucrose solution containing $NaNO_3$ and $(NH_4)_2HPO_4$. Addition of a yeast extract did not improve growth (142).

Penicillium digitatum grew poorly (first passage) or not at all in mineral-sucrose medium containing $(NH_4)_2SO_4$. Addition of autoclaved orange juice permitted good growth (53).

Penicillium expansum grew in mineral-sucrose solution containing $(NH_4)_2HPO_4$. Addition of an amino-acid mixture improved growth but yeast extract was ineffective (142).

Penicillium glaucum. A concentrate prepared from yeast greatly stimulated early growth in mineral-sucrose solution containing NH_4NO_3 (46).

Penicillium italicum grew well in mineral-sucrose solution containing $(NH_4)_2SO_4$ or $NaNO_3$ (53).

Penicillium notatum grew fairly well in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine improved growth but somewhat less than yeast extract (25).

Penicillium roqueforti grew on mineral-sucrose solution containing $(NH_4)_2SO_4$ and ten mineral supplements. Growth in seven days at 30° C. was increased by addition of a solution on which *Rhizopus suinus, Aspergillus niger* or *P. roqueforti* had grown. Beer wort or an autoclaved mixture of dextrose and ammonium tartrate was effective. Extracts of yeast or *Boletus edulis* or mixtures of pyruvic acid and glycolic acid were ineffective (65).

Penicillium rugulosum grew well in mineral-dextrose medium containing NH_4NO_3 and 1.5 per cent washed agar (77).

Pericystis apis did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates, of *i*-inositol, pantothenic acid, vitamin B_6, nicotinic acid, or lactoflavin singly or in various combinations was ineffective. Addition of yeast extract permitted growth. The growth with sub-optimal amounts of yeast extract was not improved by addition of a mixture of the six pure growth substances (121).

Philocorpa sp. produced few spores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar unless lentil extract or the inositol-free fraction of the lentil extract was added (29).

Phoma apiicola grew well in the first passage in mineral-sucrose solution containing $Ca(NO_3)_2$ but only about one-half as much in one containing KNO_3 (147).

Phycomyces blakesleeanus did not grow in mineral-dextrose medium containing asparagine. Addition of thiamine permitted normal growth (14, 106). Thiamine could not be replaced by pure lactoflavin or by 9-(dioxypyropyl)-isoalloxazine (109). Heteroauxin, nicotinic acid or nicotinamide was ineffective (89, 124, 126). Inositol and a pantothenic acid concentrate, singly or

together, were ineffective even in the presence of thiamine (89, 110, 126). Biotin was ineffective (40) as was pyridoxine (38). Pyrimidine or thiazole was ineffective but a mixture of pyrimidine and thiazole was effective (86, 124, 127). Thiochrome had little effect (51a, 123). Cocarboxylase was as effective as thiamine (48, 116, 127). Thiamine orthophosphate and thiamine disulfide were effective (51a). Ethylene chlorhydrin or pimelic acid did not replace thiamine (86) nor did heparin (51a). In a mixture of pyrimidine and thiazole the thiazole could not be replaced by *dl*-methionine, glutathione, thioglycolic acid, *S*-diphenylthiourea, thiopropionamide, allylthiourea, thiobarbituric acid, thiourea, 2-amino-4-methylthiazole hydrochloride, 2-amino-4-(*p*-diphenyl) thiazole, 1-chlorobenzothiazole, 1-mercaptobenzothiazole, 1-phenylbenzothiazole, 1-methylmercaptobenzothiazole, 5-amino-1-mercaptobenzothiazole hydrochloride (86) thiazolidine-4-carboxylic acid, or 2-keto-4-methyl-2:3-dihydrothiazole-2-benzylidine hydrozone (89). The results with other substitutes for thiazole are shown in the following table:

$$N\text{——}C\text{—}R_4$$
$$R_2\text{—}C \qquad C\text{—}R_5$$
$$\diagdown S \diagup$$
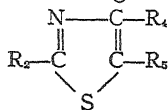
Thiazoles

Position			Activity	References
R_2	R_4	R_5		
H	CH_3	CH_2CH_2OH	active	10, 89, 116 127
H	CH_3	$CH_2CH_2OCOCH_3$	active	51a
H	CH_3	$CHOHCH_3$	inactive	10
H	CH_3	H	inactive	10, 111
H	CH_3	CH_3	inactive	111
H	CH_3	C_2H_5	inactive	10
H	CH_3	CHO	inactive	10
H	CH_3	$COCH_3$	inactive	10
H	CH_3	CH_2Br	inactive	10
H	CH_3	CH_2CN	inactive	10
H	CH_3	$CH=CH_2$	sl. active	10
H	CH_3	CH_2COOH	inactive	10, 89
H	CH_3	$CH_2COOC_2H_5$	inactive	89
H	CH_3	CH_2CONH_2	inactive	89
H	CH_3	CH_2CH_2SH (picrate or free base)	sl. active	10
H	CH_3	$CH_2CH_2NH_2$ (picrate or free base)	sl. active	10
H	CH_3	CH_2CH_2Cl (picrate)	active (10%)	10, 89

			Activity	Reference
H	CH_3	$CH_2CH_2CH_2OH$	sl. active / inactive	10 / 89
H	CH_3	$CH_3CHOHCH_3$	sl. active / inactive	10 / 89
H	CH_3	$CH_2CH_2N(CH_3)Br$	inactive	10
H	CH_3	CH_2CHNH_2COOH	active (10% or less)	82a
H	CH_3	$COOC_2H_5$	inactive	89
H	CH_3	$CH_2CH_2OC_2H_5$ (picrate)	active (3%)	89
H	CH_2OH	CH_2CH_3	inactive	10
H	CH_2CH_2Cl	CH_3	inactive	10
CH_3	CH_3	H (pheniodide)	inactive	89
CH_3	CH_3	CH_2CH_2OH	inactive	10, 89
NH_2	CH_3	CH_2CH_2OH	inactive	10
SH	CH_3	CH_2CH_2OH	inactive	111
CH_2CH_2OH	CH_3	CH_2CH_2OH	inactive	10
H	CH_3	CH_2CH_2OH (methiodide)	inactive	10

In a mixture of pyrimidine and thiazole the pyrimidine could not be replaced by nucleic acid or its acid hydrolysate nor by 15 uracils or related compounds, all of which had the pyrimidine ring, nor by 2-amino-5 methyl 1:3:4 thiodiazine hydrochloride (88). The results with other substitutes for pyrimidine are shown in the following table:

$$
\begin{array}{c}
N{=\!=\!=}C{-}R_6 \\
| \quad\quad | \\
R_2{-}C \quad C{-}R_5 \\
\| \quad\quad \| \\
N{-\!-\!-}C{-}R_4
\end{array}
$$
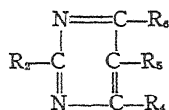
Pyrimidines

		Position		Activity	References
R_2	R_4	R_5	R_6		
CH_3	H	CH_2Br	NH_2	active	10, 86, 88
CH_3	H	CH_2NH_2	NH_2	active	10, 111, 127
CH_3	H	$CH_2OC_2H_5$	NH_2	active	10, 86, 88
CH_3	H	H	NH_2	inactive	10, 88
CH_3	H	CH_3	NH_2	inactive	111
CH_3	CH_3	H	NH_2	inactive	88
CH_3	H	CN	NH_2	sl. active	10
CH_3	H	CH_2COOH	NH_2	inactive	10
CH_3	H	CH_2CONH_2	NH_2	inactive	10
CH_3	H	CH_2NHCHS	NH_2	active—50% fairly 7% slightly	88 127 10 111
C_2H_5	H	CH_2Br	NH_2	active	89a, 114
CH_3	H	CH_3	OH	inactive	111
CH_3	H	CH_2NH_2	OH	inactive	10

CH₃	H	CH₂OH	OH	inactive	10
CH₃	H	CH₂NHCHS	OH	inactive	127
CH₃	H	CH₂OC₂H₅	OH	inactive	10, 88
CH₃	NH₂	COOC₂H₅	H	inactive	116
CH₃	NH₂	H	OH	inactive	111
CH₃	H	H	SH	inactive	111
H	H	CH₂Br	NH₂	inactive	89a
OH	H	H	OH	inactive	111
OH	CH₃	CH₂OH	OH	inactive	88
CH₂SH	CH₃	H	OH	inactive	88
C₆H₅	CH₃	H	COOH	inactive	88
C₆H₅	COOH	H	COOH	inactive	88
CH₃	CH₃	H	CH₃	inactive	88
Cl	Cl	CH₂Cl	CH₃	inactive	88
Cl	CH₃	CH₂Cl	Cl	inactive	88
CH₃	H	CH₂SO₂OH	NH₂	inactive	51a

Two compounds containing the normal thiazole and groups other than pyrimidine were found to be inactive, but were active when used with the normal pyrimidine. These compounds were 3-(4[5]-methylimidazole)-4-methyl-5-β-hydroxyethyl thiazole and 3-benzyl-4-methyl-5-β-hydroxyethyl thiazole (111). The activity of various analogs of thiamine which have substitutions either on the pyrimidine or on the thiazole ring is shown in the following table:

$$
\begin{array}{c}
\text{N} \equiv\!\!= \text{C--NH}_2 \cdot \text{HCl} \qquad\qquad \text{Cl} \\[2pt]
\quad|\ 1 \quad 6| \qquad\qquad\qquad\qquad | \\[2pt]
\text{CH}_3\text{--C}\,2 \qquad 5\,\text{C--CH}_2\text{------N------C--CH}_3 \\[2pt]
\quad\|\ 3 \qquad 4\| \qquad\qquad \|\ 3 \quad 4\| \\[2pt]
\text{N------C--H} \qquad\qquad \text{C}\,2 \quad 5\,\text{C--CH}_2\text{CH}_2\text{OH} \\[2pt]
\qquad\qquad\qquad\qquad \text{H} \diagdown \quad \diagup 1 \\[2pt]
\qquad\qquad\qquad\qquad\qquad \text{S}
\end{array}
$$
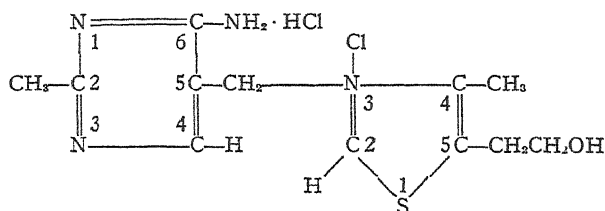
Thiamine

2	4	5	6			
(CH₃)₂CH	H	CH₂	NH₂	normal thiazole	inactive	(117)
H	CH₃	CH₂	NH₂	normal thiazole	sl. active	(117)
CH₃	H	CH₂	OH	normal thiazole	inactive	(117)
Cl	Cl	CH₂	CH₃	normal thiazole	inactive*	(88)
Cl	CH₃	CH₂	NH₂	thiazole iodide	inactive†	(10)
C₂H₅	H	CH₂	NH₂	normal thiazole	more active than methyl thiamine	(114, 117)

	2	4		5		
normal pyrimidine	H	CH₃		CH₂CHOHCH₃	sl. active (0.1)	(10)
normal pyrimidine	H	CH₃		(CH₂)₂OH	sl. active (0.06)	(10)
normal pyrimidine	H	CH₃		CHOHCH₃	inactive	(10)
normal pyrimidine	H	CH₃		H	inactive	(10)
normal pyrimidine	H	CH₂CH₂CH₃		CH₂CH₂OH	sl. active (0.05)	(117)

* Active when used with the normal pyrimidine.
† Inactive when used with the normal pyrimidine.

Three compounds in which the thiazole of thiamine was replaced by a substituted pyridine gave the following results:
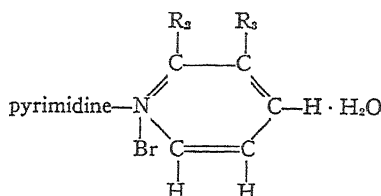
	R2	R3		
normal pyrimidine	CH_3	CH_2CH_2OH	inactive*	(84)
normal pyrimidine	CH_3	$CHOHCH_3$	v. sl. active	(118)
normal pyrimidine	H	CH_2OHCH_3	v. sl. active	(118)

* Active when used with the normal thiazole.

Addition of various plant extracts, including extracts of *Phycomyces,* to mineral-dextrose medium containing asparagine and thiamine increased the percentage of spore germination and early mycelial growth at 25° C. (80, 81, 82, 83, 85, 105, 122). This effect was not produced by various chemically pure vitamins or amino acids. Guanine and hypoxanthine were partially effective. Adenine, xanthine, and other purines were ineffective (83, 92, 93). This fungus has been used freely in bio-assays for thiamine and its intermediates (10, 15, 47, 48, 51a, 55, 125, 128, 129).

Phycomyces nitens. Numerous zygospores were formed on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar plus lentil extract. None appeared on the same medium without lentil extract. An inositol-free fraction of the lentil extract was less effective than the lentil extract (29). *P. nitens* grew poorly in mineral-sugar agar medium containing asparagine. With addition of thiamine good growth but no zygotes occurred (14). The fungus did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted luxuriant growth. A mixture of thiazole and pyrimidine was as effective as thiamine but neither intermediate alone was effective (44, 87). Autoclaving five hours at pH 10 destroyed activity of thiamine but did not destroy activity of a mixture of the intermediates (44). Addition of heteroauxin was ineffective (43).

Phymatotrichum omnivorum grew well in mineral-dextrose solution containing NH_4NO_3 and mineral supplements (132).

Phytophthora boehmeriae did not grow in mineral-dextrose solu-

tion containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were ineffective (79, 87).

Phytophthora cactorum produced no oospores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract induced formation of oospores. An inositol-free fraction of lentil extract was less effective (29). Addition of a pantothenic acid concentrate to a mineral-dextrose solution containing KNO_3 failed to induce growth (42). The fungus did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth. Thiazole and pyrimidine singly or together, were ineffective (79). *P. cactorum* did not grow in mineral-dextrose medium containing ammonium tartrate. Addition of thiamine permitted good growth (25). Addition of biotin and inositol had no effect on growth even in the presence of thiamine (31, 40).

Phytophthora cambivora did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were ineffective (79, 87).

Phytophthora capsici did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were ineffective (79, 87).

Phytophthora cinnamomi did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were ineffective (79, 87).

Phytophthora citrophthora did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth. Thiamine could not be replaced by pyrimidine and thiazole, singly or together (79).

Phytophthora colocasiae grew poorly in mineral-dextrose solution containing asparagine even on addition of thiamine (87).

Phytophthora cryptogea did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were ineffective (79, 87).

Phytophthora drechsleri did not grow in mineral-dextrose solution

containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were not effective (79, 87).

Phytophthora erythroseptica did not grow on mineral-dextrose medium containing two per cent agar and NH_4NO_3. Addition of yeast extract or thiamine gave good growth. The fungus grew in liquid medium plus amino acids and thiamine. Thiazole and pyrimidine, singly or in mixture, were not effective (44). Cocarboxylase was as effective as thiamine. This fungus was suggested for the bioassay of thiamine, and data on dry weights obtained with various quantities of thiamine were given (47, 48). No oospores were produced on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar; few were formed on the same medium plus lentil extract (29).

Phytophthora fagopyri did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine, pyrimidine and thiazole or pyrimidine permitted growth. Thiazole was not effective (79, 87). Ethyl-thiamine, thiochrome, pyrimidine or 2-ethyl-5-aminomethyl-6-amino-pyrimidine was as effective or almost as effective as thiamine. 2-methyl-5-thioformylaminomethyl-6-amino pyrimidine and five other pyrimidines were ineffective (see *Polyporus adustus*) (120).

Phytophthora palmivora did not grow in mineral-dextrose medium containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were not effective (79, 87).

Phytophthora parasitica did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth. Pyrimidine and thiazole, singly or together, were not effective (79, 87). The fungus did not grow on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar or in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth. The intermediates of thiamine or biotin methyl-ester was ineffective (31).

Phytophthora terrestria grew poorly (first passage) in mineral-sucrose solution containing KNO_3. Addition of a bios preparation from yeast produced a marked increase in growth (97).

Pilaria anomala did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted growth but ad-

dition of wheat-germ extract was much more favorable (108). Pyrimidine was nearly as effective as thiamine; thiazole was ineffective (115).   2-methyl-5-thioformylaminomethyl-6-amino-pyrimidine, 2-5-dimethyl-6-amino-pyrimidine or 2-ethyl-5-amino-methyl-6-amino-pyrimidine was as effective as pyrimidine.   Five other pyrimidines were ineffective (see *Polyporus adustus*). Thiochrome was effective but less so than thiamine (120).

Pilaria moreaui did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar.   Addition of yeast extract to the agar medium gave good growth.   Addition of thiamine was ineffective, but thiamine with an amino-acid mixture was effective.   Thiazole and pyrimidine together or pyrimidine alone was effective in place of thiamine; thiazole was ineffective (44). *P. moreaui* grew poorly (first passage) in mineral-dextrose medium containing asparagine.   Addition of thiamine or a mixture of thiazole and pyrimidine permitted good growth.   Thiazole or pyrimidine singly had no effect (115).

Piptocephalis freseniana grew in mineral-dextrose solution containing asparagine (108).

Pityrosporum ovale did not grow in mineral-dextrose solution containing asparagine.   Addition of oleic acid permitted growth which was accelerated by the further addition of pyridoxine (vitamin B_6).   A mixture of oleic acid and thiamine was more effective than a mixture of oleic acid and pyridoxine.   Thiamine or pyridoxine used singly was ineffective (7).

Plectospira gemmifera did not grow in mineral-dextrose solution containing NH_4NO_3.   Addition of yeast extract permitted good growth.   Addition of heteroauxin had no effect (43).

Pleurotus corticatus did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar.   Addition of yeast extract to the agar medium gave good growth.   Addition of thiamine was ineffective but thiamine with an amino-acid mixture was effective.   Thiazole and pyrimidine together, or pyrimidine alone, was effective; thiazole was ineffective (44).   Heteroauxin was ineffective (43).

Podospora curvula produced scanty mycelium on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar.   Addition of thiamine had no effect; addition of biotin methyl-ester permitted good growth but no fruiting; addition of biotin and thiamine resulted in numerous perithecia (31).

Polyporus abietinus (two strains) did not grow in mineral-sugar solutions containing inorganic nitrogen or asparagine. Addition of thiamine permitted growth and was almost as effective as addition of yeast extract or malt extract. Addition of biotin methylester and inositol was ineffective even in the presence of thiamine (25, 40). *P. abietinus* grew on mineral-cellulose or mineral-lignin medium containing two per cent agar (28). The fungus grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine increased growth four times. A mixture of thiazole and pyrimidine was as effective or more so than thiamine. Thiazole or pyrimidine used singly was ineffective (121).

Polyporus adustus (four strains) did not grow in mineral-sugar solution containing inorganic nitrogen or asparagine. Addition of thiamine permitted growth and was as effective or nearly as effective as yeast extract. Addition of biotin methyl-ester and inositol, singly or together, was ineffective even in the presence of thiamine (25, 40). Pyrimidine, 2-ethyl-5-aminomethyl-6-amino-pyrimidine, thiochrome, 2-methyl-5-thioformylaminomethyl-6-amino-pyrimidine or ethyl thiamine was as effective as thiamine. The following pyrimidines were ineffective:

> 2-5-dimethyl-6-hydroxy-pyrimidine
> 2-methyl-6-mercapto-pyrimidine
> 2-5-dimethyl-6-amino-pyrimidine
> 2-methyl-4-amino-5-carbethoxy-pyrimidine
> 2-methyl-4-amino-6-hydroxy-pyrimidine
> 2-6-dihydroxypyrimidine (uracil) (120)

Thiazole was ineffective (121).

Polyporus annosus did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted some growth; addition of yeast extract gave better growth than was obtained with thiamine. Effectiveness of thiamine was much increased with long periods of culture (70 days or more) (25).

Polyporus benzoinus did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted some growth in 15 days. Addition of yeast gave better growth. Effectiveness of thiamine was much increased with long periods of growth (90 days) (25). The fungus grew (first passage) in mineral-dextrose solution containing asparagine. Addition

of thiamine increased growth four times. A mixture of thiazole and pyrimidine was as effective or more so than thiamine. Thiazole or pyrimidine used singly was ineffective (121).

Polyporus fomentarius did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth which was nearly as good as that obtained on addition of yeast extract (25).

Polyporus spraguei grew on mineral-sugar-agar medium. Addition of thiamine had little effect. Growth on a thiamine-peptone agar was poorer than on potato-dextrose or malt extract agar (73).

Polyporus squamosus grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine increased growth seventy times. A mixture of thiazole and pyrimidine was nearly as effective as thiamine. Pyrimidine was partially effective but thiazole was ineffective (121).

Polyporus zonatus did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth (25). Two strains grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine increased growth up to two hundred times. A mixture of pyrimidine and thiazole was as effective as thiamine, but pyrimidine or thiazole used singly was ineffective (121).

Polystictus versicolor grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine increased growth fifty times. A mixture of thiazole and pyrimidine was nearly as effective as thiamine. Thiazole or pyrimidine used singly was ineffective (121).

Pseudopeziza ribis was tentatively listed as a biotin-deficient organism (45).

Pyronema confluens grew and fruited on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar, but addition of lentil extract improved fruiting. Inositol-free extract was less effective than lentil extract (29). Addition of auxin, hetero-auxin or thiamine to a mineral medium made with washed agar increased mycelial growth in the first thirty hours; for heteroauxin the increase was as much as 176 per cent. Similar additions to the same medium increased perithecial production for the first three days, for heteroauxin as much as forty times. No difference was visible after six days (39).

Pyronema domesticum grew and fruited on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar but addition of lentil extract improved fruiting. Inositol-free extract was less effective than lentil extract (29).

Pythiomorpha gonapodyides did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of yeast extract or thiamine to agar medium gave good growth. The fungus did not grow in liquid medium containing NH_4NO_3 and thiamine but grew with amino acids and thiamine. Thiazole and pyrimidine together or pyrimidine alone was as effective as thiamine; thiazole was ineffective (44). Cocarboxylase took the place of thiamine (47, 48). Heteroauxin was ineffective (43).

Pythiomorpha oryzae did not grow on mineral-dextrose medium containing two per cent agar and NH_4NO_3. Addition of yeast extract or thiamine permitted growth. The fungus grew in mineral-dextrose solution containing amino acids and thiamine. Thiazole and pyrimidine, singly or together, were not effective (44).

Pythium acanthicum grew poorly (first passage) in mineral-sucrose solution containing KNO_3. Addition of a bios preparation from yeast improved growth (97).

Pythium arrhenomanes did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted good growth (87). The fungus grew in a dilute mineral-dextrose solution containing NH_4NO_3 (100).

Pythium ascophallon was listed as unable to utilize thiazole and pyrimidine, singly or in mixture, but to require the thiamine molecule (45). Cocarboxylase was effective as a substitute for thiamine (47).

Pythium butleri did not grow in a concentrated mineral-dextrose solution containing asparagine. Addition of thiamine, pyrimidine and thiazole or pyrimidine permitted growth. Thiazole was ineffective. Dilution of the mineral-dextrose medium permitted growth in the absence of thiamine (87, 90, 99).

Pythium debaryanum formed oospores more freely on mineral-dextrose solution containing KNO_3 and 1.5 per cent agar plus lentil extract than on the same medium lacking the extract or on potato-dextrose agar (29). A bios preparation did not increase dry weight in mineral-sucrose solution containing KNO_3 (97).

Pythium deliense grew in dilute mineral-dextrose solution containing NH_4NO_3 (100).

Pythium graminicolum grew in dilute mineral-dextrose solution containing NH_4NO_3 (100).

Pythium hyphalosticton grew in dilute mineral-dextrose medium. Addition of thiamine had no effect (98). The fungus did not grow in a more concentrated mineral-dextrose medium (99).

Pythium indigoferae did not grow in dilute mineral-dextrose solution containing NH_4NO_3 but grew when peptone was substituted for the NH_4NO_3 (100). Addition of thiamine to the NH_4NO_3 medium was ineffective. Addition of casein, lentil, or yeast extract permitted growth to occur (98, 101).

Pythium intermedium grew in mineral-sucrose solution containing KNO_3. Addition of a bios preparation from yeast retarded growth (97).

Pythium irregulare grew in mineral-sucrose solution containing KNO_3. Addition of a bios preparation from yeast had no effect (97).

Pythium mamillatum grew in mineral-sucrose solution containing KNO_3. Addition of pure auxin *a* had no effect, addition of a bios preparation from yeast retarded growth (97). The fungus grew in a dilute mineral-dextrose solution containing NH_4NO_3 (100).

Pythium oligandrum did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of yeast extract or thiamine to the agar medium permitted growth. The fungus grew in liquid medium containing an amino acid mixture and thiamine but not in liquid medium containing NH_4NO_3 and thiamine. Thiazole and pyrimidine together or pyrimidine alone was as effective as thiamine. Thiazole was ineffective (44).

Pythium polycladon grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine, pyrimidine and thiazole, or pyrimidine permitted good growth. Thiazole was ineffective (87).

Pythium polymastum did not grow on mineral-dextrose medium containing two per cent agar and NH_4NO_3. Addition of yeast extract or thiamine permitted growth. The fungus grew in mineral-dextrose solution containing an amino-acid mixture and thiamine. Thiazole and pyrimidine, singly or together, were not effective (44). Heteroauxin was ineffective (43).

Pythium scleroteichum grew in mineral-dextrose solution containing asparagine (87).

Pythium splendens. Addition of a bios preparation from yeast had a slight beneficial effect in mineral-sucrose solution containing KNO_3 (97).

Rhizoctonia crocorum was difficult to grow on an artificial medium (20, 21).

Rhizoctonia solani grew rather poorly in the first passage in mineral-sucrose solution containing $Ca(NO_3)_2$ and not at all in one containing KNO_3 but lacking calcium (147).

Rhizopogon roseolus grew little in mineral-dextrose solution (first passage). Addition of thiamine increased growth more than twenty times. Yeast extract was somewhat more favorable than thiamine. Addition of biotin methyl-ester and inositol, singly or together, to mineral-dextrose solution containing ammonium tartrate was ineffective. Addition of thiamine and biotin or of thiamine and inositol was better than thiamine alone and as good as all three growth substances (56).

Rhizopus bovinus grew well on mineral-dextrose medium containing asparagine and agar. It grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine to the liquid medium had little effect. Addition of wheat-germ extract doubled growth (108).

Rhizopus chinensis grew in mineral-dextrose solution containing asparagine. Addition of thiamine decreased growth. Addition of wheat-germ extract increased growth (108).

Rhizopus japonicus grew in mineral-dextrose solution containing asparagine. Addition of thiamine decreased growth. Addition of wheat-germ extract increased growth (108).

Rhizopus maydis grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine had little effect. Addition of wheat-germ extract more than doubled growth (108).

Rhizopus nigricans grew in mineral-dextrose solution containing asparagine. Addition of thiamine decreased growth. Addition of wheat-germ extract increased growth (108). Addition of heteroauxin was ineffective (43).

Rhizopus nodosus grew in mineral-dextrose solution containing asparagine. Addition of thiamine decreased growth. Addition of wheat-germ extract increased growth (108).

Rhizopus oryzae grew in mineral-dextrose solution containing asparagine. Addition of thiamine decreased growth. Addition of wheat-germ extract increased growth (108).

Rhizopus suinus grew well in mineral-dextrose solution containing asparagine. Addition of thiamine inhibited growth, wheat-germ extract improved growth (104, 108). Addition of heteroauxin did not improve growth (126). Inhibition of growth by thiamine, observed for the first five days of growth, disappeared later; and some benefit from thiamine was noted. Pyrimidine and thiazole, singly or together, or cocarboxylase showed no inhibitory effect (48). Increased growth in four days at 32° C was obtained on addition of extracts of yeast or *Boletus edulis* or of beer wort to mineral-sucrose solution containing $(NH_4)_2SO_4$ and ten mineral supplements. The medium on which *Aspergillus niger, Penicillium roqueforti* or *R. suinus* had grown also was effective. An autoclaved mixture of dextrose and ammonium tartrate was effective but mixtures of pyruvic and glycolic acids were not (65).

Rhizopus tonkinensis grew in mineral-dextrose solution containing asparagine. Addition of thiamine decreased growth. Addition of wheat-germ extract increased growth (108).

Rhizopus tritici grew in mineral-dextrose solution containing asparagine. Addition of thiamine decreased growth. Addition of wheat-germ extract increased growth (108).

Rhodotorula aurantiaca grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine doubled growth but it was still poor. Addition of pyrimidine, thiazole or thiochrome was ineffective (115).

Rhodotorula aurea grew in mineral-dextrose medium containing asparagine (115).

Rhodotorula flava grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine increased growth. Addition of a mixture of thiazole and pyrimidine or of pyrimidine alone increased growth. Thiazole was ineffective; *i*-inositol and a pantothenic acid concentrate, singly or together, were ineffective (112, 115).

Rhodotorula glutinis grew in mineral-dextrose solution containing asparagine (115). *Rhodotorula glutinis* var. *infirmo-miniata* grew poorly in a mineral-dextrose solution containing asparagine even with the addition of thiamine or its intermediates (115).

Rhodotorula mucilaginosa did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine or pyrimidine permitted growth. Thiazole was ineffective (115). Addition of 2-methyl-5-thioformylaminomethyl-6-amino-pyrimidine, 2-5-dimethyl-6-amino-pyrimidine or 2-ethyl-5-amino-methyl-6-amino-pyrimidine was as effective as addition of pyrimidine. Five other pyrimidines had no effect (see *Polyporus adustus*). Thiochrome was effective but less so than thiamine (120).

Rhodotorula rubra grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine, of a mixture of thiazole and pyrimidine or of pyrimidine increased growth. Thiazole was ineffective. *I*-inositol and a pantothenic acid concentrate, singly or together, did not replace thiamine. Thiochrome was effective but less so than thiamine (112, 115). 2-methyl-5-thioformylaminomethyl-6-amino-pyrimidine, 2-5-dimethyl-6-amino-pyrimidine or 2-ethyl-5-aminomethyl-6-amino-pyrimidine was as effective as pyrimidine. Five other pyrimidines had little or no effect (see *Polyporus adustus*). Thiochrome was effective but the amounts required were larger than those of thiamine (115, 120).

Rhodotorula sanniei did not grow in mineral-dextrose solution containing $(NH_4)_2SO_4$. Addition of thiamine, of a mixture of thiazole and pyrimidine or of pyrimidine permitted growth. Thiazole was ineffective. The organism grew in mineral-glycerine solution with no added thiamine or intermediates. The glycerine was redistilled under vacuum. This torula grew on mineral-dextrose medium containing $(NH_4)_2SO_4$ and 2 per cent washed agar (26).

Rosellinia arcuata was tentatively listed as a biotin-deficient organism (45).

Rosellinia necatrix produced few spores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar unless lentil extract or the inositol-free fraction of the lentil extract was added (29).

Rosellinia thelena grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (121).

Sabouraudites gypseus grew in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect but a concentrate of rice polishings improved growth (75).

Sabouraudites radiolatus grew in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect but a concentrate of rice polishings improved growth (75).

Saccobolus depauperatus grew in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar but produced apothecia freely only on addition of lentil extract. Inositol-free lentil extract was ineffective (29). This fungus was tentatively listed as a biotin-deficient organism (45).

Saprolegnia delica was capable of unlimited growth in mineral-dextrose solution. Addition of thiamine had no effect (102).

Saprolegnia diclina did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of yeast extract permitted good growth; heteroauxin was ineffective (43).

Saprolegnia mixta grew on mineral-dextrose medium containing *l*-cystine and two per cent agar. The substitution of several other amino acids and thiamine for *l*-cystine was ineffective (44).

Saprolegnia parasitica did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine or riboflavin was ineffective. Addition of yeast extract, *l*-cystine or *dl*-leucine permitted growth. Other amino acids were ineffective (44).

Schizophyllum commune grew and fruited in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract increased fruiting (29). The fungus grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine markedly improved growth (87). *S. commune* grew very little in mineral-dextrose solution containing asparagine. Addition of thiamine increased growth over 100 times. Ethyl thiamine was as effective or more effective than the methyl thiamine. Pyrimidine was nearly as effective as thiamine; 2-methyl-5-thioformylamino-methyl-6-amino-pyrimidine or 2-ethyl-5-aminomethyl-6-amino-pyrimidine was partially effective. Six other pyrimidines were ineffective (see *Polyporus adustus*). Thiochrome was partially effective (120).

Sclerotinia cinerea did not grow in mineral-dextrose solution containing inorganic nitrogen, asparagine or amino acids. Addition of prune, apple, or peach juice or other extracts or concentrates of natural origin permitted normal growth (140). When my-

celium was used as inoculum, *S. cinerea* grew in first passage in mineral-dextrose solution containing ammonium tartrate but addition of thiamine nearly doubled growth. Addition of biotin methyl-ester and inositol had little effect even in the presence of thiamine (25, 40).

Sclerotinia libertiana (two strains). One grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$. The other made only half as much growth (147).

Sclerotium delphinii grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine, pyrimidine and thiazole, or pyrimidine permitted good growth. Thiazole was ineffective (87).

Sclerotium rolfsii grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine, pyrimidine and thiazole, or pyrimidine permitted good growth. Thiazole was ineffective (87).

Septoria apii grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine or a mixture of pyrimidine and thiazole increased growth fifteen times; pyrimidine was partially effective; thiazole was ineffective. *S. apii* grew on a mineral-dextrose medium containing asparagine and two per cent washed agar. Addition of thiamine, pyrimidine or a mixture of thiazole and pyrimidine had a small beneficial effect (121).

Septoria azaleae grew in mineral-sucrose solution containing asparagine (33).

Septoria callistephi grew in mineral-sucrose solution containing asparagine (63).

Septoria chrysanthemella grew in mineral-sucrose solution containing asparagine (34).

Sordaria fimicola did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture or thiamine with amino acids was ineffective. Good growth occurred when yeast extract was added to the agar medium (44). *S. fimicola* was tentatively listed as a biotin-deficient organism (45). The fungus produced few spores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar unless a lentil extract or the inositol-free lentil extract was added (29). *Sordaria* resembled *Melanospora destruens* in growth-substance deficiencies (31).

Sordaria longicaudata produced abundant perithecia on horse manure on which a copious growth of *Pilobolus* had developed. None was found on sterilized compost which had not previously borne a crop of *Pilobolus* (32).

Sordaria sp. produced few spores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar unless lentil extract or the inositol-free fraction of the lentil extract was added (29). *Sordaria* resembled *Melanospora destruens* in growth-substance deficiencies (31).

Spermophthora gossypii grew more vigorously on potato-extract agar than on mineral-dextrose medium containing 1.5 per cent agar and asparagine. Addition of peptone to the latter medium markedly improved growth (23).

Sphaerobolus stellatus grew in light on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar and on potato-dextrose agar but did not fruit. Addition of lentil extract induced fruiting on both media (29).

Sphaeropsis malorum grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$ (147).

Sphaerulina trifolii grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine, pyrimidine and thiazole, or pyrimidine permitted good growth. Thiazole was ineffective (87).

Sporodinia grandis produced negligible growth on mineral-dextrose medium containing 1.5 per cent agar and KNO_3 (3). *S. grandis* grew poorly in mineral-dextrose solution containing KNO_3. Addition of a bios preparation from yeast improved growth (97). The fungus grew on mineral-dextrose medium containing asparagine (108).

Sporormia intermedia did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture or thiamine with amino acids was ineffective. Growth occurred when yeast extract was added to the agar medium (44). The fungus was tentatively listed as a biotin-deficient organism (45).

Sporotrichon beurmanni grew in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine or of a concentrate of rice polishings markedly improved growth (75).

Sporotrichon councilmanni grew in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect but a concentrate of rice polishings improved growth (75).

Sporotrichon gougeroti (2 strains) grew in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had little or no effect but a concentrate of rice polishings was effective (75).

Sporotrichon schenckii grew in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine improved growth but a concentrate of rice polishings was more effective (75).

Stereum frustulosum did not grow on mineral-sugar medium containing agar and $(NH_4)_2SO_4$ or asparagine. Addition of thiamine permitted growth. Addition of thiamine to a peptone medium markedly increased growth until it equaled that on potato-dextrose or malt agar (73).

Stereum gausapatum. Considerable differences between growth rates of various strains on potato-dextrose agar and on Difco malt agar were noted. Transfers from old agar cultures through a few successive passages grew a few millimeters and ceased to grow, grew in a lobed fashion or grew at a uniform rate with an even margin. Successive transfers from the most vigorously growing portion of the mycelium grew better than the immediate parent mycelium (35).

Sterigmatocystis violae grew well in the first passage in mineral-sucrose solution containing KNO_3 or $Ca(NO_3)_2$ (147).

Stigmella platani-racemosae. Sporulation occurred in juice expressed from leaves of *Platanus racemosa* and filtered sterile. Abundant spermatia were produced on sterile filter paper wet with the juice (130).

Stigmina platani. Sporulation occurred in juice expressed from leaves of *Platanus racemosa* and filtered sterile. Abundant spermatia were produced on sterile filter paper wet with the juice (130).

Syncephalastrum cinereum grew in mineral-dextrose solution containing asparagine (108).

Thamnidium chaetocladioides did not grow in mineral-dextrose solution containing NH_4NO_3. Addition of yeast extract per-

mitted good growth. Addition of heteroauxin was ineffective (43).

Thamnidium elegans grew in mineral-dextrose solution containing asparagine (108). Addition of thiamine had no effect (87).

Thielavia basicola produced delicate inconspicuous growth on corn meal, prune, oat, pea, potato, carrot, and bean meal agar and few perithecia. Addition of unheated water extracts from *Thielaviopsis basicola, Cladosporium fulvum, Aspergillus umbrosus, Thielaviopsis paradoxa,* yeast or Taka-diastase markedly improved growth and production of perithecia. The heated extracts were ineffective (54).

Thielaviopsis basicola was unable to utilize pyrimidine or thiazole, singly or together; it required the thiamine molecule (45).

Thraustotheca clavata did not grow on mineral-dextrose medium containing NH_4NO_3 and two per cent agar. Addition of thiamine, riboflavin, an amino-acid mixture or thiamine with amino acids was ineffective. Growth occurred when yeast extract was added to the agar medium (44). *T. clavata* was tentatively listed as a biotin-deficient organism (45).

Tilletia horrida grew poorly (first passage) in mineral-dextrose solution containing asparagine. Addition of thiamine or a mixture of pyrimidine and thiazole increased growth forty times. Pyrimidine or thiazole used singly was ineffective (121).

Tilletia levis grew on mineral-dextrose medium containing asparagine and two per cent washed agar. Addition of thiamine had no effect (121).

Tilletia tritici grew poorly in mineral-dextrose medium containing asparagine and two per cent washed agar. Addition of pyrimidine, thiamine, or a mixture of thiazole and pyrimidine improved growth slightly (121). Defago quoted by Blumer and Schopfer found this organism to require molecular thiamine.

Torula cremoris did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect. A mixture of thiamine and nicotinamide was ineffective. Good growth was obtained on mineral-dextrose medium containing agar, thiamine, and peptone (91).

Torula fermentati grew poorly in mineral-dextrose solution containing asparagine. Addition of a mixture of thiazole and pyrimidine increased growth. Thiazole or pyrimidine used singly was ineffective (91).

Torula hansen grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (91).

Torula kefyr did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect. A mixture of thiamine and nicotinamide was ineffective. Good growth was obtained on mineral-dextrose medium containing agar, thiamine, and peptone (91).

Torula laurentii grew poorly in mineral-dextrose solution containing asparagine. Addition of a mixture of thiazole and pyrimidine increased growth. Thiazole or pyrimidine, used singly, was ineffective (91).

Torula rosea did not grow in mineral-dextrose solution containing asparagine. Addition of pyrimidine or a mixture of pyrimidine and thiazole permitted growth. Thiazole was ineffective (91).

Torula sanguinea did not grow in mineral-dextrose solution containing asparagine. Addition of pyrimidine or a mixture of pyrimidine and thiazole permitted growth. Thiazole was ineffective (91).

Torula sphaerica grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (91).

Torulopsis candida grew poorly in media lacking growth substances. Growth was stimulated by *Penicillium rugulosum* or its products. *T. candida* did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates was ineffective but addition of yeast extract permitted growth (121).

Trachysphaera fructigena did not produce oospores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar unless lentil extract was added. Inositol-free lentil extract was ineffective. The organism produced few conidia on agar medium without lentil extract and it required lentil extract for good production of oospores in liquid culture (29). *T. fructigena* was tentatively listed as a biotin-deficient organism (45).

Trametes cinnabarina did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth. Addition of biotin and inositol was ineffective even in the presence of thiamine (25, 40).

Trametes serialis did not grow on mineral-dextrose solution containing ammonium tartrate. Addition of thiamine was as effective or nearly as effective as yeast extract. Addition of biotin and inositol was ineffective but growth with all three substances was better than with thiamine alone (25, 40).

Tricholoma albobrunneum grew little in mineral-dextrose solution (first passage). Addition of thiamine permitted some growth but yeast extract gave over twice the yield obtained with thiamine. Addition of thiamine, biotin methyl-ester or inositol, singly or in combination, had no effect on growth in mineral-dextrose solution containing ammonium tartrate (56).

Tricholoma imbricatum grew slightly in mineral-dextrose solution (first passage). Addition of thiamine increased growth but growth with yeast extract was over twice that obtained with thiamine. Addition of biotin methyl-ester or inositol to a mineral-dextrose solution containing ammonium tartrate was ineffective, and neither increased the effectiveness of thiamine (56).

Tricholoma nudum did not grow in mineral-dextrose solution containing ammonium tartrate. Addition of thiamine permitted growth. Addition of biotin methyl-ester and inositol was ineffective even in the presence of thiamine (25, 40).

Tricholoma pessundatum grew little in mineral-dextrose solution (first passage). Addition of thiamine increased growth over four times. Yeast extract was more effective than thiamine. Addition of biotin methyl-ester and inositol, singly or together, to mineral-dextrose solution containing ammonium tartrate had no effect and did not increase the effectiveness of thiamine (56).

Trichophyton crateriforme grew slowly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect, but a concentrate of rice polishings improved growth (75).

Trichophyton discoides grew poorly on a dextrose-peptone agar. Addition of mycelium of *Actinomyces albus* or of thiamine markedly improved growth. It did not grow on a mineral-dextrose medium containing asparagine or nitrate and agar. Addition of thiamine to these media was ineffective (51).

Trichophyton interdigitale did not grow in mineral-sugar medium containing amino-acids. Addition of crystalline thiamine, inositol, a highly concentrated preparation of pantothenic acid, or

crude lactoflavin permitted growth. Some combinations of these substances were more effective than the single substances (61).

Trichophyton rosaceum grew slowly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine or of a concentrate of rice polishings improved growth (75).

Trichosporon beigelii grew poorly in a solution of sodium chloride, ammonium lactate, Na_2HPO_4, asparagine and sugar. Addition of thiamine had no effect, but a concentrate of rice polishings permitted good growth (75).

Typhula variabilis was listed as a thiamine-deficient organism (45).

Ustilago avenae grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (119).

Ustilago bromivora (two strains) grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had little effect (119).

Ustilago hordei grew poorly in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had little effect (119).

Ustilago levis grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had little effect (119).

Ustilago longissima grew in mineral-dextrose solution containing asparagine. Addition of thiamine, of a mixture of thiazole and pyrimidine, or of pyrimidine increased growth. Thiazole had no effect (119).

Ustilago nuda grew in mineral-dextrose solution containing asparagine. Addition of thiamine or its intermediates had no effect (119).

Ustilago scabiosae did not grow in mineral-dextrose solution containing asparagine. Addition of thiamine permitted growth, a mixture of thiazole and pyrimidine was effective but less so than thiamine. Addition of pyrimidine or thiazole singly was ineffective. Of eight different pyrimidines and five different thiazoles only the pyrimidine and thiazole intermediates of thiamine were effective (8). This is contrary to an earlier report that the mixture of pyrimidine and thiazole was ineffective (119).

Ustilago tritici grew in mineral-dextrose solution containing aspara-

gine. Addition of thiamine or its intermediates had little effect
(119).

Ustilago violacea did not grow in mineral-dextrose solution con-
taining asparagine. Addition of thiamine or a mixture of
pyrimidine and thiazole permitted growth. Pyrimidine or thia-
zole was ineffective. The strain from *Dianthus deltoides* showed
less response to a mixture of pyrimidine and thiazole than to
thiamine (119).

Ustilago zeae grew in mineral-dextrose solution containing aspara-
gine. Addition of thiamine had little effect (119).

Valsa ceratophora grew some in a first passage in mineral-dextrose
solution containing ammonium tartrate when mycelium was used
as inoculum. Addition of thiamine more than doubled growth
and yeast extract was still more effective (25).

Valsa pini did not grow in mineral-dextrose solution with am-
monium tartrate. Addition of thiamine, of biotin methyl-ester,
of inositol, of thiamine and inositol or of biotin and inositol was
ineffective. *Valsa* grew with addition of thiamine and biotin and
still better with addition of thiamine, biotin and inositol (25).

Ventura inaequalis. An extract of *Penicillium* increased mycelial
growth and greatly increased the formation of perithecia on oat-
meal agar. The autoclaved extract was less effective than that
filtered sterile (143).

Vermicularia sp. grew well in the first passage in mineral-sucrose
solution containing $Ca(NO_3)_2$ or KNO_3 (147).

Xylaria arbuscula grew well in sucrose-mineral solution containing
NH_4NO_3 (11).

Xylaria hypoxylon grew poorly in mineral-sucrose solution contain-
ing NH_4NO_3, much better with asparagine and well with peptone.
(11). This fungus grew poorly in mineral-dextrose solution
containing ammonium tartrate. Addition of thiamine increased
growth five times (25).

Xylaria polymorpha grew in mineral-sucrose solution containing
NH_4NO_3, but growth was more than doubled when asparagine
was used as a nitrogen source (11).

Zygorhynchus dangeardi grew in mineral-dextrose solution con-
taining asparagine (108).

Zygorhynchus exponens showed no response to thiamine (14).

Zygorhynchus heterogamus grew in mineral-dextrose solution con-

taining NH_4NO_3. Addition of heteroauxin was ineffective (43).

Zygorhynchus moelleri produced few zygospores on mineral-dextrose medium containing KNO_3 and 1.5 per cent agar. Addition of lentil extract greatly increased the number of zygospores. An inositol-free fraction of lentil extract was less effective than the lentil extract (29). The fungus grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

Zygorhynchus sp. #113 grew in mineral-dextrose solution containing asparagine. Addition of thiamine had no effect (87).

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Continued increase in value attached to THE BOTANICAL REVIEW as a permanent record of botanical progress has provoked preparation of increasingly more comprehensive reviews. As a result, the REVIEW has been published during the current year at a greater rate than in previous years. Continuation of this rate would involve expenses which, without an increased subscription rate next year, would exceed those permitted by its budget. Therefore, in order to avoid such increased rate, it becomes necessary to discontinue publication during August and September. The entire pagination for the volume, however, will be in excess of 600 pages.

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TAXONOMY AND PHYLOGENY

W. B. TURRILL
Royal Botanic Gardens, Kew, Surrey, England

PART II

CONTENTS

TAXONOMIC AND PHYLOGENETIC CONCEPTS AND CRITERIA

Theories of Evolution

The general theory of evolution, *i.e.*, the origin and development of all kinds of organisms, extant or extinct, from pre-existing kinds by mechanisms which can be discovered and studied, is accepted now by practically all biologists. It follows that knowledge of phylogeny is a possibility and that, given all the relevant data, a complete phylogenetic scheme can be made. How such a scheme can be expressed, whether it would be too complicated to use in taxonomy, or how far it could be so used, are among the more important problems which have to be discussed below.

There is still considerable doubt as to the relative importance of the various theories proposed as explanations of the mechanism or mechanisms of evolution. A majority of botanists appear to favour Darwinian as opposed to Lamarckian views, judging from those

who have expressed their opinions on this matter in publications. The original theory of mutations and the modern experimental methods in the study of heredity were both introduced by botanists De Vries and Mendel. It is doubtful that theories as to the means of evolution have as yet much affected either taxonomic practice or phylogenetic theories. It is perhaps of some significance that those who have made the causes or means of evolution a main study have not produced general phylogenetic schemes. This is true of Lamarck, Darwin and De Vries. Lotsy published three large volumes essentially on plant phylogeny; he then investigated causal evolution and, as a result, forcibly condemned all phylogenetic schemes, including his own.

It is unnecessary here to discuss the well known theories of the mechanisms of evolution associated with the names of Lamarck, Darwin, De Vries and others. Lotsy (271), for example, fully outlines them. For variations, additions and some more heterodox views, others (279, 329, 330, 508, 362) may be consulted. There are, however, a few modern works dealing with evolution in which conclusions are expressed that are less generally known but which have a more or less direct bearing on our present thesis.

Berg (41) contends that evolution has been determined by law (nomogenesis) rather than by selection from the accidents of variation; that is, evolution follows a definite course which is shaped by both external (geographical) and internal (autonomic) causes. One important statement for which one would like more detailed evidence is that "in the origination of new geographical forms (species, sub-species, nationes) a vast number of individuals inhabiting a certain geographical area are simultaneously involved in the production of new characters". Certain kinds of distribution in plants, when studied comparatively, *e.g.,* in *Colchicum* and *Abies,* suggest that mass changes may occur through the whole or a large part of the geographical range of a group approximately at the same time. Turrill (448, 449) has suggested the term *hamagenesis* for this hypothesis.

Clark (98, 99), working on zoological data, believes that palaeontological and embryological evidence leads to the conclusion that "in its broader features the development of animal forms took place by *concurrent evolution*", that there was "simultaneous development of some representative or representatives of all, or practically

all, of the phyla or major groups of animals at the time of the very first appearance of life", and that "all of the major groups of animals were formed at the same time as the result of following different developmental paths from the primitive single cell. . . . There is no evidence of any kind which would lead us to suppose that any one of the major groups was derived through any of the others". "The process leading to and resulting in the first appearance of some representative or representatives of each of the major groups may be known as *eogenesis*". "Through eogenesis the ground is prepared for the growth of the evolutionary trees. Therefore the picture that we get shows a whole forest of evolutionary trees of widely different sizes each of which arose from a seed formed and planted by the process of eogenesis". Classes within a major phylum are also usually distinct without intergrades, and probably they arose "in the early embryonic stages of the animal types concerned more or less immediately after the fixation of the structural complex characteristic of the phyla". In a less and less extreme form abrupt discontinuities may be followed into orders, suborders, families, genera and species.

Rosa (364) has developed the concept of *ologénèse* from zoological studies. The word is derived from ὅλος, entire, and is attached to a theory according to which "l'évolution se produit suivant des lignes ramifiées dichotomiquement et chaque espèce est prédéterminée dans l'espèce précédente comme un individu est prédéterminé dans l'oeuf". Rosa classes his theory with those postulating an internal cause as a predeterminant, not merely of variation but of the direction of evolution. He particularly stresses "L'hypothèse que la ramification des lignes phylétiques est due à des dédoublements qui doivent nécessairement subir les espèces en conséquence de la constitution à laquelle ètait parvenu leur idioplasma (ou plasma propre) au bout de son évolution précédente". Unfortunately, the work lacks clarity and is largely occupied with generalities rather than with actual proof and examples of hologenesis. Since phyla, *i.e.*, species and through them higher taxonomic groups, are by hologenesis formed in pairs by the entire disappearance of the parent phylum, the continued existence of organisms of lower rank has to be explained by the subsidiary hypothesis of different idioplasms (even if they have originated dichotomously from a common source) having "une différente

potentialité phylogénétique". In this way it can be explained "pourquoi dans les diverses lignées (*phyla*) l'organisation a atteint des niveaux si différents". There is much vagueness in the presentation of the main hypothesis, some of the statements made do not hold for plants, and it is not known how far the concept of dichotomy, in any strict sense, can be applied to the known facts of evolution, in view of modern cytogenetic knowledge of mutations and hybridity.

Some of the philosophical aspects of phylogeny, mainly German, are discussed at length by Beurlen (45).

A zoologist, Przibram, proposed the theory of *apogenesis* which postulates that, instead of supposing phylogeny to be represented by a tree, "all the facts would be explained more easily by supposing that there existed, at the beginning, many organised substances developing side by side into species, each of the latter passing through stages more and more advanced without actual relationship of descent between the different species" (74, 343). Since hybridization is being increasingly shown to be an important factor in evolution in plants, it is unlikely that such a hypothesis will be found to have much value in the study of plant phylogeny.

The modern study of heredity and evolution is largely experimental in nature with, one is glad to note, an increasing linkage with field studies. Most phylogentic schemes have been prepared in museums, herbaria, laboratories or libraries. This suggests the need for closer co-operation of those working on different lines of research. How far it is advisable to separate macro-evolution from micro-evolution is questionable, but the terms are sometimes useful in discussing the problems of phylogeny. Phylogeny of the major groups has, on the whole, been studied by methods different from those used, especially most recently, in studying phylogeny at about the species level. The problem arises as to whether major group characters have in some way a different basis in the genotype from that of genes responsible for characters of less systematic worth. As Darlington has shown (122), the "genotype must be supposed to embrace three elements, nucleus, plastids and cytoplasm. All of these are subject to particulate inheritance and particulate variation. The nucleus in addition is subject to a super-particulate chromosome variation to which the others presumably are not. The particles in the nucleus are genes; those in the plastids and cyto-

plasm may perhaps be treated more rigorously if we also think of them as genes—plastogenes and plasmogenes". This is an exhaustive classification, since one can not conceive of genes in the cell wall, food reserves or waste products, though these may influence gene expression in characters through alteration of pH of the cell sap, *etc.* There remain, however, other possibilities on which a taxonomist would like the opinion of cytogeneticists. Thus, it seems certain that there are very different rates of mutation for different known gene-loci. Is the explanation simply that, for example, family characters of the taxonomist are inherited by means of non-mutable, or very rarely mutable, genes, while varietal characters are connected with genes that mutate more easily? Is there some position effect controlling this difference? Position here would mean not merely position relative to other genes in a linear series but also a position to exposure to or protection from influences or agents causing mutation, such as shallower or deeper embedding in some protective chromatin layer. The tendency in genetics now-a-days is to regard "unit characters" as a mere device for expressing empirically obtained results and to consider gene interaction within whole chromosomes or within the whole genom as the only unit basis of inheritance. Goldschmidt (167) even denies the existence of the gene. Thus, he says: "All points taken together suggest strongly that the chromosome is the actual hereditary unit controlling the development of the Wild type, that purely steric changes at the individual points of its length produce deviations from Wild type which may be described as mutations, though no actual Wild-type allelomorph and therefore no gene exists". To a certain extent such a view appears to depend on the definition accorded to the word gene, and there is little doubt that for the practical purpose of describing experimental and observational results the term gene will long continue to be used.

Can, then, family and other characters of a high taxonomic grade have a basis of genes which have become relatively isolated from the influence of other genes and are thus responsible for characters which are uninfluenced or little influenced by modifying genes which in turn may be relatively highly mutable? A very different possibility is that high grade taxonomic characters are due primarily to genes whose alleles would result in early death of the organism or in such characters that it could not perpetuate itself. Such non-

perpetuation might be due to unbalance of basic and, in a sense, specialized organization rather than to the unfavorableness of single characters. A somewhat similar effect would result if genes responsible for a high-grade taxonomic character had pleiotropic or pseudopleiotropic effects (171), one of which affected vital characters. It is very probable that some combination of the above possibilities may account cytogenetically for taxonomic grading in so far as this is valid—and much of it certainly is. The whole matter is commended to the attention of cytogeneticists interested in the broader problems of evolution. On the taxonomic side, a careful analysis of the characters used in a selected number of groups of different hierarchical levels with synthetic tabulations of the results might throw much light on our problems. What is needed is a cytogenetic explanation of Church's words (95) : "The older the factors of organization the more deeply they are impressed in the constitution of all races to come". To the taxonomist there seems to be much matter for thought and investigation in this sentence.

Gates (156) reached the conclusion that higher organisms exhibit two contrasted types of characters which he designates karyogenetic (nuclear, mutational) and organismal (recapitulatory, orthogenetic), respectively. While it is doubtful that these two categories, at least to any marked degree, correspond with taxonomic grading of characters, their possible relevance should be considered in any comprehensive survey of taxonomic characters— a survey which, it is again emphasized, is badly needed.

Lotsy (275) says that the real problem of evolution is: How did the great phyla arise and why do they, after a shorter or longer time, fade out and are they replaced by others? There is no evidence of any of the great phyla having sprung from a pre-existing one. On the contrary, more and more evidence is accumulating of the extreme antiquity of all of them. He concludes that the great phyla probably arose independently from unicellular organisms and have originated through plasma crossing before uniparental (maternal) transmission of the cytoplasm replaced the earlier biparental transmittance. This agrees, in part, with Clark's theory of eogenesis, outlined above.

The discussion in the last few paragraphs raises one very important issue which must be faced. Are, or how far are, taxonomic

groups artificial devices, or do they, or to what extent do they, represent groupings in nature? The most diverse opinions are expressed, often very dogmatically, on this question. It is stated, on the one hand, that all taxonomic categories are at best merely convenient fictions; on the other hand, that they represent, if they be real, the classification of Nature herself. These views are combined in any of the four possible ways with the views that a natural classification must be phylogenetic or based only on the largest possible number of characters. Many taxonomists appear to agree, more or less, that the major groups, down to families or even genera, are artificial to varying degrees, but contend that species are real groupings found as such in Nature. "Man makes orders, families, and genera, nature makes species." Others contend that all taxonomic categories are man-made and that Nature makes only individuals. It would appear that in some groups of animals and plants Nature has not been too clear in her construction of individuals, and psychologists even recognize dual or multiple personalities in *Homo sapiens!* Questions arising from the reality or unreality of species are discussed by Turrill (447). Difficulties arise partly from the use of ambiguous terms like "natural", "artificial", "real", and so on, and partly from a lack of knowledge of the general meaning and principles of classification. Definitions can be made by which any and every group is natural or real and others by which any and every group is artificial or unreal. In biological taxonomy a group is natural in the sense that it is composed of a finite number of organisms, a population; it is artificial in the sense that the taxonomist selects the characters by which he delimits the group and places it in the taxonomic hierarchy. In these respects, it is difficult to see any difference between any of the taxonomic categories. The extremely practical device of the taxonomic hierarchy means that the higher the rank of the group, the fewer are the characters shown in common by all its members; the lower, the larger the number of common characters of all members of a group. This holds, must hold logically, for any given series, but it holds from one series to another only to the degree that the groups on any one taxonomic level are equivalent from series to series. It is generally agreed that groups are frequently not equivalent in the degree of (usually morphological) distinctness from series to series. For example, genera in Orchidaceae, Compositae and Labiatae are more

finely drawn than in, say, Ranunculaceae. This discrepancy raises
other issues which, however, need not detain us. We can logically
avoid it here by keeping within one series.

Species Concept

In any accepted series, say, orders, families, genera, species
(plural), varieties, we usually find that a greater number of criteria
separate the species one from another than separate the genera,
families or varieties.   That is to say, most often, though there are
exceptions, the term species is given to groups of organisms formed
on the basis of the greatest number of differences between the
groups.   Often there are more morphological group differences
between species than between families, genera or varieties.   Other
and particularly some modern workers emphasize ecological, genet-
ical or cytological differences.   Further, it seems that a high degree
of correlation is shown by the different kinds of criteria at the taxo-
nomic species level.   Nevertheless, in other groups there is less
correlation, and the taxonomic species then depends on the criterion
selected or emphasized—morphological distinctness, geographical
isolation, ecological limitations, sterility, etc.   The criteria and
their correlation are essentially sense data, and their use (selection,
emphasis, etc.) in the formation of classes (species, genera, etc.)
is dependent on the taxonomist.   The greater the correlation be-
tween a greater number of criteria, the more natural is the class.
In this sense only taxonomic species are usually more natural than
genera.

Another argument, with a good deal of truth behind it, is to state
that so many groupings, essentially different, are included in one
taxonomic category, e.g., species, that any attempt to define the
categories in their present use or misuse or to debate whether they
be natural or artificial, is a waste of time until it be determined how
many categories are needed to avoid ambiguity, what criteria should
be used to define them, and what names should be given them.   At
present, on one basis or for one purpose, a species may be a good
species; from another viewpoint it may be equally valid to consider
it a variety or subspecies of another species (cp. 180).

If the taxonomist insist that a group must be monophyletic he is
introducing a phylogentic criterion which adds to the characters
to be correlated, assuming that he has evidence for determining
monophyly apart from correlation of characters.

Watkins (480) concludes that "The idea of species is valid in so far as it emphasizes that differences exist which are unlike those commonly found in an interbreeding population, and often larger. It is artificial to the extent that it includes in one category distinctions of totally different natures and origins, and presupposes a sharp line, where none can be drawn, between the large and the small".

Robson (361) expresses the following opinions: "The proposal is frequently made that the concept of species and the systematic method of specific diagnosis should be given up in favour of some other system preferably based upon recognition of the method in which the differentiated characters are variously combined. The rejection of the systematists' concept does not, however, seem to be implied in these conclusions. Ill-defined as they may be and of varying dimensions, a certain tendency to character-groupings of a certain stability is fairly recognizable, and such grouping requires designation, the only difficulty being the line to be drawn between species and variety. From the point of view of classification, it seems desirable to retain that admittedly arbitrary device than to adopt a system of elaborate symbolic representation. At the same time there is no doubt that, if the systematist were to adopt some method of expressing character-groupings and combinations as an adjunct to his traditional method, it would illustrate the structural relationships of allied forms in a very useful manner".

Rabel (345), in an exceedingly interesting paper which should be read by all biologists, after pointing out that "A clear formulation of the species problem can never be attained unless the two factors Resemblance and Descent are carefully kept apart", suggests, in outline, a decimal system for organisms, exemplifying its form and possible use from the angiosperms. A taxonomist may doubt the practicability of such a scheme applied to all organisms because of the enormous number of characters which would have to be included, the impossibility of memorizing masses of symbol combinations, and the great risk of writing, typing or printing wrong letter or figure symbols with little chance of a reader detecting errors, but its possibilities require investigation. Marsden-Jones and Turrill in *Silene* (289), Turrill in *Glaucium* (451) and Chaytor and Turrill in *Clypeola* (87) have used schemes with a somewhat similar basis for the study of problems at and below the species level.

An excellent summary of the facts and theories regarding the constitution of species is given by Cuénot (118).

Characters

It is impossible to lay down general laws as to what is or is not a single or unit character. Pleiotropism and pseudopleiotropism, additional to the ordinary difficulties of classifying characters, make simple genetical tests by themselves, even theoretically, of no greater value in taxonomy than conclusions from comparative morphology in deciding this question. Thus, Marsden-Jones and Turrill (292) found in male (female sterile) *Ranunculus acris* that a wide range of characters, affecting vegetative, floral and reproductive organs were always inherited together in the now hundreds of living plants studied, the assumption being that they were controlled by a single gene. Yet the low height and general habit, the clawed thick leaves, the small flowers, the narrow more numerous petals, and the reduced carpels would all be referred to as separate characters in any morphological-taxonomic description of the plants. On the other hand, a study of all variations within a group will give a minimum figure value for single characters as accepted by the systematist. The same phenotypic character may, of course, be due to different genoms, especially such absence characters as glabrousness and albinism. We can not by any known method determine a maximum of characters from either a systematic or a genetical standpoint, but by widening observations and experiments on an ever-increasing number of samples and by the use of statistical tests, the chances of finding a breakdown in a supposedly single character are greatly increased if the character be composite. Till reasonable proof is obtained to the contrary, a character has to be considered single. Thus, to take a very common example, a simple plane outline shape is usually considered a single character in descriptive botany, but it may be proved to be composite genetically, as by varying independently in length and breadth.

The success of selection of characters for the practical purposes of classification can be judged pragmatically. There is, however, a danger in subjectively choosing characters to support a proposed phylogenetic scheme and ignoring others which lend no support to it. At least every known character should be given its due in

publication. All characters which can be made available must be weighed as accurately as possible.

There comes next the exceedingly difficult question of weighting of characters of supposed phylogenetic value. Lutjeharms (277) shows that the distinction between essential and unessential characters, given great prominence in phylogentic studies, dates back to Aristotle. The test of judicious weighting for the purpose or purposes of a practical classification must again be pragmatic. Does the relative evaluation, combined with previous selection of characters, result in a classification permitting precise and easy determination and/or an arrangement in which organisms alike in most of the available characters are classed near together, *etc.*? On the other hand, in trying to determine phylogeny on the basis of character resemblances and differences, weight is nearly always given to some character as having greater phylogenetic importance than others. How is this phylogenetic importance determined? When based on palaeontological evidence or, for lower taxonomic grades, on cytogenetic data, it may well be sound. In the absence of such basis, one can frequently detect an argument in a circle. Removing all verbose camouflage, a commonly used argument reduced to its essential terms is that a group is primitive or advanced because its members have primitive or advanced characters, while the characters are primitive or advanced because they occur in a primitive or advanced group. It can not be too strongly stated that such a circle must be broken before the inferences become valid. It can be broken only objectively, by independent evidence reasonably proving, *i.e.,* so far as such matters can be proved, either that the group is or the characters are primitive or advanced.

Series

A very large number of progressive series have been made in the various groups of plants, particularly series showing more or less graduated changes (character clines) in one organ or what is conveniently considered as a unit organ. This study of organogenesis or morphogenesis has intrinsically much to recommend it, since it brings to light many unknown facts, unrealized connections and correlations, and suggestions for further research. The difficulty is to know how correctly to read the series morphogenetically, whether up or down, or to start somewhere between the two ends and to

read in two directions. For the Spermatophyta, Engler (140, 141) and Diels (131) give numerous examples of progressive series, and Zimmermann (506, 507) discusses the interest and importance of Merkmalsphylogenie. Wilson's account of the phylogeny of the stamen (493) is an example of recently published work on organogenesis based mainly on anatomy. The theory of phyllome development, as stated by Domin (133), is another example. Where it is possible, as by palaeontological evidence, by certain distinction of vestigial from rudimentary structures, by clear evidence of progressive specialization, *etc.*, to determine the morphogenetic trend, there still remains the essential problem of uniting the different trends or series to determine the phylogeny of groups as composed of whole organisms. The reticulate nature of such trends rather than their parallelism is often obvious.

The very difficult problem of the use of abnormalities in tracing organogenesis must be briefly considered. Worsdell (501) holds "that there can never have been, so far as vascular plants are concerned, more than three categories of organs [*i.e.*, three main subdivisions of the plant-body] in the past from which all modern structures have descended, *viz.* stem, leaf, and root." Indeed, accepting, as he does, the phyton theory, even the stem "is built up solely by the bases of the leaves". Teratology is one of the methods which can be used "to determine the origin, from one of the three categories of organs, of any structure whose present nature is doubtful, and whose mere appearance is perhaps wholly misleading". The method has to be used with care and constantly supplemented by comparative and other evidence, but "In very many cases the so-called freaks and monstrosities represent reversions or harkings-back, in one form or another, to an ancestral condition". Obviously, any valid evidence which will determine a more primitive condition of an organ must greatly assist in the correct formation and reading of a morphogenetic series of that organ.

It is perhaps desirable to give two or three examples of organogenetic series, but considerations of space demand excessive brevity. The 40 to 50 genera of the Ranunculaceae, with some exceptions, *e.g.* the *Anemone* and *Aquilegia* groups, are fairly sharply distinguished morphologically. It is possible to make a number of organogenetic series, such as:

<div align="center">

Herbaceous ⇌ woody habit
Actinomorphy ⇌ zygomorphy
Petalous ⇌ apetalous
Numerous carpels ⇌ one carpel per gynoecium
Carpels free ⇌ carpels united
Numerous ovules ⇌ one ovule per carpel

</div>

Yet these series are not parallel, taking all genera into consideration. If, for example, the series be read from left to right: *Clematis* is advanced in having a woody, climbing, habit, in being most often apetalous, and in having one or two ovules, only one of which ripens to a seed in an achene fruit, and primitive in showing actinomorphy, numerous carpels, and free carpels. On the other hand, *Ranunculus* is advanced in having only one ovule per carpel, but primitive in the other five series. *Nigella* is advanced in having united carpels, relatively advanced in having a definite number of carpels, and primitive in the other four series. *Delphinium* is advanced in having zygomorphic flowers and few (usually 3) to one carpel per flower, and primitive in the other four series. There are other organogenetic series besides the six listed, and including these it is possible to show that every genus in the family shows a mixture of advanced and primitive characters.

In the Gramineae, the Bambuseae show morphological characters, especially of the flower, which are usually regarded as primitive (see 19, 24, 25, 47, 208, 342). Some of Avdulov's cytological results and deductions have, however, been criticized (147, 346, 323, 97). Flovik gives reasons for assuming that the original basic number of chromosome units was 5, not 12 as assumed by Avdulov, and that "The higher basic numbers now generally occurring must therefore be regarded as secondarily balanced basic numbers". Exact cytological data for the Bambuseae are few, but the $2n$ numbers 48, 54 and 72 have been reported (227, 502). These numbers do not suggest 5 as the basic number for the tribe, and if Flovik be right, then cytologically the Bambuseae can not be regarded as primitive. It is highly desirable that more bamboos be cytologically examined. The Gramineae are of particular interest from our immediate point of view because Vavilov's theory of homologous variation was originally based largely on studies within this family. Arber (19) also gives numerous examples of paral-

lelism and repetition for both normal and teratological characters in the family.

My colleague, Mr. C. E. Hubbard, gives me the following striking examples of parallel series in the Gramineae. Reduction, perhaps also multiplication, in the numbers of flowers per spikelet must have occurred in various lines, and the one end result of one floret per spiklet has been used in a very artificial manner in classification.

In *Agropyron* (Triticeae) there are, in different species, from many to two florets per spikelet.

In *Eragrostis* (Eragrosteae) there are from ten to over 100 (in *E. tremula* Hochst.) to one to three (in *E. habrantha* Rendle).

Sporobolus is essentially *Eragrostis* with the florets reduced to one per spikelet, but on account of this reduction is placed in the tribe Agrosteae by Bentham and by Hackel.

In *Lolium* (Festuceae) from ten to two florets occur per spikelet.

In *Danthonia* (Aveneae) from ten to two florets occur per spikelet.

In *Deyeuxia* there is only one floret per spikelet, but there seems little doubt that the genus is polyphyletic, since several distinct series from as many genera can be traced leading to it. Indeed, the whole tribe Agrosteae (*sensu* Hackel) is probably highly artificial in the sense that if all possible characters were taken into account and that of one-flowered spikelets not weighted, all the genera composing it might naturally be placed in other tribes.

Awn development provides other series. In grasses awns of the lemma (flowering glume) may be produced by the apex of the lemma being three or more lobed and the central lobe produced into an awn, as in *Danthonia,* or by the middle nerve continuing from the back of the lemma as an awn, as in *Avena* and *Agrostis*. These two very different types occur even in the same tribe in existing classifications of grasses. Further, for each type, series can be traced from well developed, geniculate awns to straight bristles to small central lobes or minute dorsal projections, even in one genus, as *Danthonia* and *Agrostis* (336).

In addition, parallelisms, either as graduated series or as contrasting, presumably allelomorphic, characters are known in grasses for the following (*inter alia*): habitat—high mountain *versus* lowland, coastal *versus* inland; habit—stoloniferous or creeping *versus*

caespitose or erect, weak or robust *versus* normal; glaucous *versus* green colour of vegetative parts; variegated *versus* concolorous green; glabrous *versus* hairy sheaths and/or culms; degree of density of inflorescence; large or small spikelets *versus* normal size; variously coloured (green, pale, yellowish, purple, *etc.*) *versus* normal coloured spikelets; glabrous *versus* hairy spikelets; chasmogamous *versus* cleistogamous florets.

The Bryophyta have many characters which can be arranged in series but these series are often not parallel with one another. For example, in the Hepaticae, we find:

$$Thallus\ simple \rightleftarrows thallus\ complicated$$
$$Thallus\ dorsiventral \rightleftarrows thallus\ radial$$
$$Thallus\ not\ foliaceous \rightleftarrows thallus\ foliaceous$$
$$Perianth\ absent \rightleftarrows perianth\ present$$
$$Sporangial\ receptacles\ absent \rightleftarrows sporangial\ receptacles\ present$$
$$Sporangium\ sessile \rightleftarrows sporangium\ stalked$$
$$Sporangium\ without\ elaters \rightleftarrows sporangium\ with\ elaters.$$

There are numerous other morphogenetic series which, like those just quoted, criss-cross in larger or smaller taxonomic groups. In all probability some at least of these series are duplicated or even multiplied more than once phylogenetically, *i.e.,* they represent two or more similar lines of evolutionary change which have occurred independently of one another. For example, Gates (157) instances several lines for the development of elaters in the Hepaticae. The Marchantiales can be arranged in a series from *Riccia* with simple thallus to *Marchantia* with a highly complex thallus. Cavers (81) and Bower (52a), amongst others, accepted the series as an evolutionary one showing increasing complexity of gametophytic structure. Goebel (166) gives reasons for considering the series as a reduction series. Bower, in a later publication (58), after considering Goebel's views, concludes that "the balance of probability seems now to point to the simpler types having resulted from reduction in both of their alternating phases, rather than as themselves being primitive". In the Musci similar series can be arranged as for the Hepaticae, both for gametophytic characters and for those of the sporophyte.

From these examples it is obvious that if a conclusion is to be reached on: *a*) whether a graded series of characters indicates, as

arranged, a morphogenetic series; *b*) how such a series should be read—from left to right, from right to left, or in a di- or polychotomous manner; and *c*) if such series can be built up into a phylogenetic scheme and if they can how such a scheme is to be read, the characters have to be weighed and then evaluated from a phylogenetic standpoint. The latter process is difficult to keep entirely objective, since it means weighting or loading with the risk of bias in favour of a desired tip of the scales.

The following general principles have been used:

a) Palaeontological evidence gives relative primitiveness of groups.

b) Cytogenetic data indicate, or prove, how groups have originated, and from which other group or groups.

c) Ontogeny is supposed to repeat phylogeny, more or less.

d) More extreme specialization of structure and function are considered as evidences of a higher stage of evolution within the group, unless there is evidence of reduction. Conversely, organisms more closely approaching the general form or forms of the next higher taxonomic category are considered relatively primitive.

e) A graded series is read on the basis that members showing greater or more resemblances to a group accepted as lower in the evolutionary scale are the more primitive.

f) Orthogenetic, and sometimes teleological, trends are accepted as indicating the direction of evolution.

g) Constancy of correlation of a large number of characters in members of a group suggests that the group is relatively monophyletic, and the degree and kind of breakdown of such correlation may indicate the direction of evolution. A constant character has, in taxonomy, generally meant one found in all or nearly all members of a group, *i.e.*, in a logically natural system one of a number of characters showing high correlation. More rarely it has been used as the opposite of plastic, *i.e.*, a character not easily modified in the course of its development by modification of environmental conditions.

These general principles are sometimes combined and the evidence is then much stronger; sometimes they are subdivided and the evidence is then correspondingly weaker. Thus, Wernham (482), for the dicotyledons, considers "economy in production of the several items comprising reproductive organs" [under *f* above]

and "progressive adaptation to the reception of insect-visitors" [under *d* above] as "the fundamental guiding principles in the progressive evolutionary history of the dicotyledonous flower" and that the main tendencies subserving these two principles are increased conspicuousness (enlargement, aggregation), zygomorphy and fusion of parts.

Arber (17) points out that "The meaning of reduction-processes in evolution has been rendered unnecessarily obscure by the misleading terminology coined by morphologists. The use of the word 'economy' in connexion with reduced features in flower construction, has been peculiarly unfortunate, because it has carried with it unwarranted implication of purposefulness. No one would claim that the loss of hair in the elderly human being is an adaptation for the purpose of economizing in brushes—on the contrary, we recognise it at once as a symptom of failure in vitality due to age. And it seems reasonable that the same common-sense explanation should apply to racial losses and reductions". The warning against a terminology involving a too facile assumption of teleological explanations is opportune. The substituted explanation, however, ignores all problems of adaptation and specialization and, as a widely applicable general principle, also ignores the great mass of evidence in favor of natural selection.

Organogenetic series, whether considered as examples of progression or reduction, are usually based on morphology, but the physiological aspect has also to be considered. Thus, many of the examples considered by Haberlandt (179) in his work on physiological plant anatomy are or can be arranged in series similar to those claimed as organogenetic.

Before leaving the subject of character series one protest seems desirable. The making of such series and the study of the evolutionary history of organs or of single characters should not be termed phylogeny—Merkmalsphylogenie of Zimmermann (506, 507). It can be termed organogenesis or morphogenesis and the term phylogeny reserved for the evolutionary history of phyla in the sense of any taxonomic unit.

More detailed lists of principles for classification or indicators of phylogeny have been given by various authors. Many of these have been compiled with no more than shallow consideration of the truth of many of the statements which are made and without stating the

evidence, or without stating it in such a way that can be checked and re-evaluated. Many of the lists can be traced Engler, Hallier (often via Senn) and Bessey.

Swingle (417) says there is "rather uniform agreent on the following principles".

"1. Plant relationships are up and down genetic lines, a these must constitute the framework of phylogenetic taxonomy. This will naturally form a branching but not reticulate structure.

2. Some evolutionary processes are progressive while others are regressive.

3. Evolution does not necessarily involve all organs of the plant at one time or in the same direction. One organ may be advancing while another is stationary or retrogressing.

4. Evolution has generally been consistent, and when a particular progression or regression has set in, it is persisted in to the end of the phylum.

5. In any phylum the chlorophyll-bearing plants precede the chlorophylless ones. Saphrophytes are derived from independent forms and parasites usually from saphrophytes among the lower plants, and from independent forms among the flowering plants.

6. Usually, structures with many similar parts are more primitive, and those with fewer and dissimilar parts are more advanced.

7. Among seed plants the stem structure with collateral bundles arranged in a cylinder is more primitive than that with scattered bundles.

8. In most groups of seed plants woody members have preceded the herbaceous ones.

9. In most groups of seed plants erect members have preceded the vines.

10. Perennials are more primitive than biennials and biennials are usually more primitive than annuals.

11. Scalariform vessels are more primitive than vessels with round pits.

12. The spiral arrangement on the stem and of the floral leaves precedes that of the opposite and whorled type.

13. Simple leaves are more primitive than compound leaves.

14. Historically, leaves were first persistent (evergreen) and later deciduous.

15. Among the seed plants the netted venation of leaves is more primitive than the parallel venation.

16. The many-parted flower is the more primitive, the type with few parts being derived from it, and the change is accompanied by a progressive sterilization of sporophylls.

17. A condition in which the perianth consists of like segments is more primitive than one in which sepals and petals are unlike each other.

18. Flowers with petals preceded apetalous ones, the latter being derived by reduction.

19. Polypetalous flowers are more primitive than gamopetalous ones, the latter being derived from the former by symphysis.

20. Regular flowers preceded irregular ones.

21. Spirally imbricate floral parts are more primitive than those that are whorled and valvate.

22. Hypogeny is the primitive structure and from it perigyny and epigyny have been derived.

23. Numerous carpels represent a more primitive condition than few carpels.

24. Separate carpels represent a more primitive condition than united carpels.

25. Axile placentation preceded parietal and central placentation of ovaries.

26. The presence of numerous stamens indicates a more primitive condition than that of a few stamens.

27. Separate stamens preceded united stamens.

28. Evolution in angiosperms is believed to have proceeded from seeds with two seed coats to those with only one.

29. The primitive seed contains endosperm and a small embryo, the advanced type has little or no endosperm, with the food stored in a large embryo.

30. A straight embryo is usually more primitive than a curved one.

31. The solitary flower is more primitive than the inflorescence.

32. Bisexual flowers preceded unisexual flowers.

33. The monoecious condition is earlier than the dioecious.

34. Simple and aggregate fruits preceded multiple fruits.

35. The same evolutionary phenomena have often been repeated as separate occurrences in different parts of the plant kingdom, *e.g.,* loss of chlorophyll, loss of petals, stamens and carpels, acquisition of fleshy texture in fruits and of various types of thorns, change

from simple to compound leaves, from erect to prostrate habit, and from hypogynous to perigynous or epigynous insertion of floral parts, and lateral union (symphysis) of petals, stamens and carpels.

36. In determining the closeness of relationship between two families or other groups, it is usually best to compare with each other the more primitive, or basal, members of each group, rather than those that are simplified by reduction or those that are most highly specialized."

There is a high probability that for both plants and animals transmigration from water to land gives definite directions of evolution for certain major groups. For vascular plants the discovery of adequate material which led to the establishment of the Psilophytales gives valuable evidence for the evolution of the Pteridophyta, not only by giving a relative starting point for the phylogeny of the group but also by providing phylogenetically early stages for morphogenetic studies of both vegetative and reproductive organs, though in the fossil forms these are known only for the sporophyte generation. At the other end of the scale of vascular land plants it seems clear from geological evidence that the angiosperms were the last of the great groups to appear. Hence, changes towards characters peculiar to the angiosperms may with some justification be read as progressive, the more so that they are now the dominant group ecologically over much of the land surface of the globe. In the same way for the animal kingdom, the mammals form the last of the great groups to appear in the geological succession. The much greater relevant palaeontological evidence for groups of animals than for groups of plants makes it possible to consider the primates as the "highest" group of mammals. The placing of man as the most highly evolved of all primates may be justified palaeontologically but also accords with human conceit, and one wonders how far phylogenetic schemes for animals are subconsciously influenced by "progress" being evaluated from an anthropomorphic standpoint.

Some authors have attempted to distinguish between morphological, fortuitous, or constitutive and adaptive, biological, or non-constitutive characters. The former are said to have relation neither to the environment nor to any biological function; the latter are in direct relation to some vital function or advantage and therefore more liable to change or exposure to the action of natural selection.

Darwin (125) admitted the distinction to some extent, and Wernham (482), Diels (131) and Sprague (409, 236) have accepted it. Sprague refers to the distinction as follows: "Two corollaries have been drawn: that 'the occurrence of several common fortuitous characters in a series of plant-forms is valid evidence of their mutual affinity'; and that 'a group of plants may share a number of biological characters in common without being therefore closely related' (482). A possible objection to the acceptance of the two categories is that in many cases it may be a matter of opinion whether a particular character is or is not connected with the general mode of life of the plant or with some special function. On the whole, however, there is not much difficulty in separating characters which have no apparent connexion with function from those which definitely have such a connexion; and experience has shown that classifications based mainly on the former category exhibit greater correlation of characters than those based on the latter." Vesque (468, 469) introduced the term "épharmonique" for directly adapted characters, contrasting them with characters of "filiation", the latter alone having a value in phylogenetic studies.

Scott (386) takes exactly an opposite point of view and one which does not seem to have been fully considered by more recent writers. He says: "Another question which has been much discussed is that of the supposed distinction between morphological and adaptive characters. Personally, I do not believe that any such distinction exists. . . . On a previous occasion I was at some pains to show that certain 'typical' morphological characters, on which the distinction of great classes of plants is based, were adaptive in origin, and even that their constancy is due to their functional importance [Darwin and Modern Science, 1909]. This applied in particular to the pollen-tube and the seed, which were considered in relation to fossil evidence".

A rather interesting problem, which has been little or not at all discussed in detail by taxonomists, arises over the concept of "conservative" characters, which, it is usually stated, are the most useful for classification and most valuable in phylogenetic studies. For plants, and especially for vascular plants, it is frequently added that the reproductive organs are more conservative than the vegetative. Yet, to take the carpel as an example, on any old or new theory of carpel morphogenesis there must have been far greater morpho-

genetic evolutionary changes in carpels than in any vegetative organ considered throughout the Spermatophyta.

Tuzson (459) gives as a criterion of relative age of two groups the size of the gaps separating the subordinate groups in each. Thus, he considers the monocotyledons as an older group than the dicotyledons, since the smaller groups of monocotyledons are separable into series and families which show greater gaps on the whole than those in dicotyledons.

It has been stressed by some zoologists that certain and sometimes large groups of animals are distinguished by a common pattern or organization which, because of the large number of physiologically interconnected parts, can not be supposed to have originated more than once. This gives, it is further stated, very strong proof that the units of the group are monophyletic. This criterion appears to be of more restricted value in the study of plant phylogeny. Asa Gray (169), however, says: "A natural classification in botany aims to arrange all known plants into groups in a series of grades according to their resemblances, in all respects, so that each species, genus, tribe, order, *etc.,* shall stand next to those which it most resembles in all respects, or rather in the whole plan of structure. For two plants may be very much alike in external appearance, yet very different in their principal structure." The exact meaning of whole plan of structure is not very clear, but in these days, when holism and organism are stressed by philosophers, botanists might well give more consideration to unity of plan and its possible phylogenetic significance [see also below, under sub-heading Gross Morphology].

It is accepted by many of those taxonomists who aim at a phylogenetic system that any group must be monophyletic, at least in the sense that its constituent taxonomic units all arose from only one group of equivalent rank. The hybrid origin of certain species, and possibly of some higher groups as well, rather hinders absolute application of this principle. Sprague (236, 410) appears to regard monophyletic groups as the only natural ones—at least at and above the family level in existing angiosperms. On the other hand, Hutchinson (230), in his phylogenetic scheme and classification, definitely retains groups he considers polyphyletic, *e.g.,* Asterales and Euphorbiales. Phylogenetic taxonomists should agree as to

whether or not (proved or postulated) polyphyletic groups can be accepted in a phylogenetic classification.

One great attraction of phylogeny is that in one sense it gives a dynamic viewpoint from which taxonomic units can be considered. It is, however, doubtful how far the taxonomic units in general use—species, genera, families, *etc.*—are suitable for such treatment, since they are necessarily regarded as relatively static, as in the application of the rules of nomenclature. In evolutionary studies it may be better to replace them by, or at least to supplement them with, lineages, lineage-groups, lines of evolution, *etc.*, as suggested by Faegri (143) for species. Thus, he says: "a taxonomical-phylogenetic line of evolution is a sequence of generations, the individuals of which descend from the individuals of the preceding generation and within each generation group themselves according to the law of probability with regard to all essential features, and further, form a closed sphere of combinations, reacting avitally or incompatibly with all other spheres of combinations with which it comes into contact. The species is the momentary realization of such a line."

Anderson and Ownbey (12) conclude from studies of closely related species "that differences between such species are to be sought not in any one character but in harmoniously integrated tendencies (genetic coefficients) expressed more or less throughout the entire organism". They develop a simple mathematical notation "for expressing the resulting mathematical hiatus between two species" and discuss, *inter alia,* the application of estimates of genetic coefficients to "the determination of phylogenetic patterns".

The view that tendencies have as much value in tracing phylogeny as fixed or group constant characters, has been expressed by a number of writers. Arber (17), for example, says: "It seems to me that the tendency to progress in a certain definite direction is as much an inherent character of a given race, as are the features of its chemistry or morphology". Wernham (482) constantly refers to tendencies shown by groups of the Sympetalae or in groups supposedly ancestral to them.

We must now turn to a brief consideration of the kinds of data used in reconstructing phylogenies and often also in classifications not professing to be phylogenetic. For convenience a number of sub-headings are used.

DATA USED IN CLASSIFICATION AND PHYLOGENETIC STUDIES

Gross Morphological

We have seen that so-called natural systems were well established, without reference by their authors to phylogeny, by the formation of classes to contain organisms having in common as many as possible of the studied characteristics, those considered being, with very few exceptions, gross and external morphological. Correlation of morphological characters, resemblances and differences in common, with a certain amount of weighting, largely based on tradition, is still the basis of classification in nearly all groups of plants, except for certain special classifications. Reference may be made to Greenman (170) and his discussion of morphology as a factor in determining relationships.

Turrill (456) has pointed out that morphology, either plant or animal, nowadays includes a good deal more than the mere study of shape. Anatomy and much of cytology, ontogeny, embryology, palaeontology and genetics are or involve morphology. "Even within morphology in a very strict sense the taxonomist is forced to consider matters which involve physiology. The degree of correlation of characters and the constancy or plasticity of characters are so closely related to internal and external factors that the taxonomist must, to a certain extent, consider the physiological working of these last. . . . even the useful classification into morphological and physiological characters (attributes) is artificial, in the sense that it is purely a device of the biologist".

The occurrence, in various degrees, of plasticity complicates the taxonomic and phylogenetic use of all characters, but in a classification based entirely on morphology, only phenotypic characters can be used, and there are many examples in taxonomic literature where one knows, or strongly suspects, that different taxonomic groups have been formed for phenotypes of one genotype with the implication, at least, that they represent different genotypes. The very considerable morphological plasticity of certain species, *e.g., Plantago major*, on different soils under the same climate has been proved by Marsden-Jones and Turrill (291) in the transplant experiments of the British Ecological Society at Potterne, Wilts. For many purposes the study and even classification of phenotypes of one genotype is essential, but when they are uncritically included

in a classification with phenotypes having different genotypic bases, the results are misleading.

In the higher taxonomic divisions, *i.e.,* phyla, families, *etc.,* morphological criteria are still the only or most satisfactory ones for classification, though these often form an indissoluble combination with physiological characters. Since there is an enormous number of morphological characters, however one limits the term, taxonomists choose for use those which they consider essential or likely to give the clearest classification or that most easy to use. The phylogenist usually weights his characters, in theory, according to accepted principles, some of which have been discussed above. Turrill (456) says: "That taxonomists often choose (abstract) the characters they use as diagnostic for their groups and give them, more or less arbitrarily, higher or lower taxonomic values has one important practical bearing which is not always recognized. Often several, equally valid, classifications can be made of the same organisms, entirely on morphological attributes. In all probability one of these will be more in accord with cytological, genetical, *etc.,* classifications than the others". It follows that some one or other morphological classification is most likely to give the best basis for a general classification of the widest possible use, because morphological characters are, in general, easier to use than others. At the same time it is true that the classification of many plant groups, particularly in the angiosperms at the family level, has now been subjected to severe tests, and, as Crow (116) says, the characters used "are such as have been found by long experience to give a harmonious scheme and bring together organisms which are alike, as it were, to the core. Artificial arrangements have been tried again and again, only to be abandoned because they do not give a coherent system". The great value of a classification based essentially on gross morphology is that it enables correlations of the characters studied to be easily determined.

Smith (403), discussing the principal divisions of the Bromeliaceae, the largest family of angiosperms entirely limited to the New World, into a capsular series and a baccate series, gives an excellent example of the way in which morphological data are frequently used to indicate phylogeny. It should be noted that his conclusion depends on acceptance of a particular view of carpel morphogenesis, on correlation of morphological and ecological-phytogeographical

characters, and on an assumption. The systematic division of the family into a capsular and a baccate series is very widely accepted by taxonomists. In the former series the seeds are appendaged, in the latter unappendaged and the fruits have inferior ovaries. The sentences to which attention is particularly drawn are: "The morphological evidence indicates that the capsular series is the more primitive and gave rise to the baccate. Since carpels, sepals and petals evolved from leaves, the more they become fused the more advanced stage of development they indicate. In the capsular type the three carpels, though joined to form a compound ovary, split apart at maturity, while in the baccate they never split naturally but are irregularly ruptured by extreme age or outside agents. In the capsular type the carpels have remained distinct from the floral axis, leaving the ovary nearly or quite superior, while in the baccate type the carpels have fused with the inverted axis making the ovary inferior. Both these contrasts would denote the baccate series as the more advanced. The third contrasting character between the two series, the presence or absence of seed-appendages, is not so easily interpreted. Other things being equal, one might reason that the appendages were an acquisition and denoted advance, but in the light of the other contrasts it seems more likely that they were lost in the development of a baccate type. For with dispersal obtained through birds and animals eating the fruit, there would be no function for seed-appendages and they would tend to disappear". The entire baccate series has a more extreme development of epidermal absorptive scales. The geographical evidence supports, on the whole, the conclusion that the capsular series arose in the Andes and extending eastward gave rise to the baccate series.

In the future development of morphological classification two outstanding improvements seem highly desirable. Firstly, that the morphological categories themselves be subjected to critical investigation and, if it be found necessary, to drastic revision. This has been much stressed by Thomas (430, 431, 432). Saunders's theory of the polymorphism of carpels, whether accepted or not, points to the same necessity (376). Secondly, there is need for a full consideration of how far quantitative methods can be introduced advantageously into the morphological basis of taxonomy (*cp*. 282, 283). There seems to be considerable scope for co-operation between taxonomists and biometricians. Many of the statistical meth-

ods in vogue (145, 438, 393) are unsuited, in their existing forms, for taxonomic work, and solutions of certain important problems of a quantitative nature have apparently never been attempted by biometricians, or at least never been published.   Anderson, in various publications (2, 5, 11, 12, 13, 14, 15), has made noteworthy attempts, in part with colleagues, to devise diagrammatic and mathematical methods to express taxonomic differences, degrees of divergence and convergence, and probable phylogenetic relationships.   His methods are well worth close study, constructive criticism and further extension.   Excellent discussions of correlation will be found in Robson (361) and Robson and Richards (362).

Morphological studies have very largely been concerned with the structure of individual organs which are arbitrarily considered more or less independently from one another and from whole organisms for purposes of study.   In classification and in phylogenetic studies the unity of plan of the whole soma and life-history is, however, of considerable importance.   Interaction of parts is so close that modification of one must frequently involve modifications of others, or lethality (witness the almost innumerable lethal genes recorded), or at least disadvantages result.   Reference may be made here to Fisher's theory of dominance (146).   Hence, general unity of plan "involving homology of the different organs and systems, is a more valuable criterion of relationship than the presence or absence of any individual character could be, since it is found to extend over large groups and enables large series of organisms to be brought into a harmonious scheme" (116).   Many of the biological facts on which the theory of holism (406a) is based are almost truisms, though the present writer feels that the explanation offered is unsatisfactory, since it seems to amount to little more than that holism is in some way caused or controlled by Holism.

Natural systems for plants have been based almost entirely on morphological resemblances and differences.   Usually, in phylogenetic schemes they are expressed as ramifying or, more rarely, reticulate systems.   To the extent that these schemes do or can express the facts, the whole facts and nothing but the facts, they must be accepted as showing genetic affinities, and the classification on which they are based is, to the same extent, explicable on

evolutionary theories. It remains that historically phylogeny is based on natural classification, not classification on phylogeny. Certain modifications of this statement may be necessary for small groups of plants whose phylogeny and classification have been based on modern research not, or not entirely, of a morphological nature.

Anatomical

Anatomy (histology) is morphology with a compound microscope, but it has developed its own technique, its own outlook on general problems, and, above all, to a certain extent, its own postulates. It will be generally agreed that all aspects of plant structure and development must be considered in formulating a natural or a phylogenetic classification. Some anatomists claim that anatomical features are not infrequently more conservative than those of gross morphology and are therefore to that extent of greater use in tracing phylogeny or organogenesis. For example, vascular bundles may persist after external organs have become highly vestigial or obsolete. Vesque (468, 469) has emphasized the value of anatomy in taxonomic and phylogenetic research. Tipps (439) has given a valuable summary of the lines of evolution of stem structure, more particularly for the secondary xylem of angiosperms, as accepted by most plant anatomists. References to earlier work are given in this paper. He makes a claim which, if substantiated, is extremely important, since it may enable a break to be made in the primitive group—primitive character circle discussed above. The following are his words: "From this brief description of the methods of the anatomist, it can be seen that these lines of specialization are not based on any system of angiosperm classification, but have been formulated independent of any preconceived notion that this or that group of gymnosperms has given rise to the angiosperms; or any preconceived idea that the Ranales or the Amentiferae are primitive. Consequently any suggestions which the anatomist makes as to phylogeny will be unbiassed, and therefore of correspondingly greater value than would be the case were not his methods founded independent of classification." Stem anatomy points to the Magnoliales as a relatively primitive group of the angiosperms (439, 176). There is no doubt that anatomical characters often show an exact correlation with gross morphological and other characters and thus fit into a scheme of either logically

natural or phylogenetic classification. Solereder (407) gives numerous examples of this. Fritsch (149) has summarized the major uses of anatomy in classification. Papers, with numerous references, by Odell (328), Edwards (139) and Chalk (83) may also be consulted.

Chalk (83) notes that "the structure of wood is probably more conservative than that of the flower, and it is not surprising to find that the minor floral differences by which many species are distinguished from one another are not reflected in the wood; thus the woods of different species of a genus are often indistinguishable. Differences between genera are usually clearly defined in the wood, and where they are absent it can often be shown that the taxonomic distinction is open to question". He gives examples showing that wood anatomy can suggest improvements in classification. One danger in using wood anatomy in classification is that wood usually means secondary wood, and the stressing of criteria derived, at least, mainly, from the secondary wood of trees and large shrubs may give results statistically biassed against herbaceous groups and the herbaceous parts of woody groups.

Record (353) outlines problems of wood anatomy and emphasizes that it is a science in a formative state. He believes that "Properly developed it will profoundly effect the orderly classification of plants, will revise paleobotany, and make clearer the lines of descent in the practical field . . . ".

In the Thallophyta many organisms are microscopic in size and their morphology is of necessity anatomical. In the Bryophyta anatomical details occupy a large part of descriptions and have a corresponding importance in classification. In the Pteridophyta anatomy, both of the vegetative and of the reproductive organs, has an enhanced importance because of the available evidence of anatomy in fossils and of its use in ontogeny. The same is true for the Gymnospermae.

A new and important line of research has been the comparative study of pollen grains of existing and fossil plants. This has particularly been developed for existing plants by Wodehouse (494, 495, 496, 497, 498, 499; see also 340). There seems no doubt that pollen grain characters give data as valuable for classification as spores in fungi and other plant groups. From the phylogenetic standpoint, by assuming that the pollen grain characters of gymno-

sperms are primitive to those of angiosperms, it is possible to conclude with Wodehouse that the one-furrowed grain is a primitive character in Saururaceae, Piperaceae, Chloranthaceae and Magnoliaceae, all of which families have been considered by various authors as primitive. The one-furrowed grain is not found in Salicaceae, Juglandaceae, Betulaceae, Fagaceae and Casuarinaceae. The grains of these families show unmistakable signs of reduction and apparently trace their origin to some of the higher dicotyledons.

Attention may be called to the interesting convergence in the development of pollinia in Orchidaceae, Asclepiadaceae, Leguminosae (Mimosae and Acaciae) and Chlaenaceae (401). The adherence of pollen grains in tetrads is also recorded in a wide range of families and genera: Ericaceae, Epacridaceae, *Juncus, Luzula, Anona, Drimys, Jussiaea, Periploca, Jacquinia, Fourcroya* and *Typha.*

Wilson (493) has considered the phylogeny of the stamen, mainly on the basis of its anatomy.

Warming (478) has published an account of the systematic value of the anatomical structure and orientation of the ovule. Engler's use of ovule characters in parts of his classification is now usually considered to give an artificial result.

Physiological and Biochemical

Turrill (456) has pointed out that there is no valid *a priori* reason why a classification of plants should not be made entirely on physiological characters. Functions are as varied as organs and show comparable stability or plasticity. They are, however, generally less easy to observe and to define precisely. "The close linkage so often obvious between form and function and the fact that subjects such as cytology, ecology, and genetics, have fundamentally important, though often little understood, physiological aspects, must eventually lead the taxonomist to give more consideration to physiological attributes".

Up to the present it has been biochemical rather than directly functional criteria that have been used in taxonomy. Essential oils, resins, latex, glucosides, alkaloids, *etc.* show in their occurrence some degree of correlation with other characters, and their presence or absence has been used in classification. An excellent

review of the subject is given by Molisch (308) who concludes that, while we are only at the beginning of phytochemical knowledge, the phylogenetic value of phytochemistry is already considerable. Especially can the chemistry of plant substances and their distribution suggest the correctness or otherwise of phylogenetic schemes based on morphological or other criteria. He points out that a definite chemical substance may appear in a single species, in several species of the same genus, in a single genus, in several genera of one family, in an entire family, in two to many related families, in two to many unrelated families, or in large divisions of the plant kingdom.

McNair has published a number of papers showing the distribution of certain chemical substances in the families of flowering plants and its relation to climate (see 299 and earlier references there). Climatic conditions, presumably on the basis of natural selection, have a major influence on the distribution of plants containing certain substances—fats, volatile oils, alkaloids, *etc.* A general conclusion is, however, reached: that for the tropics, and perhaps for all climates, the more highly organized the plant the more highly organized are likely to be its chemical products. The trends suggested in McNair's tables and figures are not always very convincing, since they show very many and large irregularities.

The detailed work of Reichert (355) on the starches is particularly important, since he shows that it is possible to identify many plants by their starch grains, or, alternatively, to name the plant from which a given sample of starch grains was obtained, by utilizing a combination of characters (shape, size, markings, chemico-physical properties) of the starch grains alone.

Roberts and Doyle (360) state that in the conifers individual and habitat variations in the pH of the leaves is small (about 0.3 from the average, and often less). Seasonal changes are also small. All the conifers examined lay within the pH range of 3 to 6, but some complete families (Pinaceae, Araucariaceae, Taxaceae) are characterized by a narrower range. The large indefinite Cupressaceae, as well as the Podocarpaceae, are not so characterized, but the more natural affinities of most of the smaller sections within them are expressed by definite pH groupings. They conclude that "there is in the conifers a very strong tendency for

natural affinity to be expressed in similarities of metabolism which are reflected in the correspondence of the pH range of the related groups".

Redfield (354) has pointed out that in zoology "Classical evolutionary theory is based primarily on morphological data. These data are formulated under the name of comparative anatomy; a mature body of doctrine in which the rules of the game are well established and agreed upon. . . . One of the rules of the game in comparative anatomy states that analogous structures do not count. Analogies are physiological resemblances, and it results that much of comparative physiology is ruled out *a priori* from the game of tracing evolutionary relationships". While phylogenists may dislike the word game in this connection, the statement raises problems of general biological importance, since we know that many physiological functions are fairly directly genetically controlled, and that form and function are always intimately related. The following additional paragraphs from Redfield are so relevant to our subject that they are worth quoting: "If the distribution of chemical peculiarities among the natural groups of organisms is to be given an intelligent interpretation, we must first develop some satisfactory criteria by which to judge what resemblances are significant in an evolutionary sense and what are not. We need some body of chemical doctrine, similar to that which embryology has given to the morphologists, by which to judge our findings. We must know not only what substances occur here and there, but also how they come to be where they are, from what they are made, and how their occurrence is determined".

"A not unlimited number of basic configurations appear to be used repeatedly by animals and plants in forming complex molecules of the greatest importance. The various groups of organisms can be distinguished by their abilities to construct, transform, or utilize these substances".

"There are certain limitations which the chemical nature of organisms places on the nature of variation (see Pantin: Jour. Linn. Soc. Zoology 37: 705. 1932). An organism can never be infinitely plastic. Its variation is limited by the variation of its chemical substances. It must occur step by step, each step as discrete as those which separate the species which chemists designate as different compounds". Illustrations are given which show "the

multiplicity of the changes which must simultaneously occur in any successful evolution of function".

Other relevant references are to papers by Blackman (49) on the carbohydrate production in the higher plants from the point of view of systematic relationship, and by Rosenthaler (365) on the general connections between plant biochemistry and systematics.

The use in phylogenetic studies of the anatomy of traumatic (wound) structures and reactions has been much discussed (see 28, 243, and references in these).

Serology has been developed in the past few decades, following advances in bacteriological technique. Reference may be made to Vigano (470) and Chester (90) for practical methods. Turrill (456) summarizes the position as follows: "In its taxonomic application this [serology] is the injection into an animal of protein or other organic material of known specific origin. The blood serum reacts to the extraneous matter and after being cleared will cause a visible reaction when added to a solution or suspension of what is chemically the same as the original or closely related material. Several methods, varying in detail, have been used (precipitation, agglutination, anaphylaxis, complement-fixation). For taxonomic and phylogenetic studies in plants the precipitation and agglutination methods have been chiefly employed, proteins obtained from seeds being the injected material. Serum from an animal into whose blood protein from a given plant *A* has been injected is added to a solution or suspension of material from a plant *B*. The degree of precipitation or agglutination is taken as a degree of relationship between *A* and *B*. . . . That serological characters must be considered in future taxonomy is certain and improvements in methods and in presentation of results are likely to make serum-diagnosis more useful than it has been up to the present. . . . Recognizing that serum-diagnosis can yield characters which may be used in classification there remains to consider whether such characters are of any greater value for phylogenetic theories than other, for example, morphological, characters. Moritz (312) has urged that a high diagnostic value must be given to proteins on account of their occurrence in all organisms and of the structural complication of the protein molecule. This last, he thinks, makes convergence improbable, and, therefore, the theory of parallelism of serological activity and physiological significance can not be

assumed.   Whether improved biochemical technique and direct determination of the proteins will not eventually replace the indirect determination of protein relationship, it is difficult to say. Logically, in spite of Moritz's contentions, it seems difficult to give protein characters any higher taxonomic value than other characters which may, after all, be considered as manifestations of protoplasmic (genic) activity".   Chester *et al.* (91) have shown, on the other hand, that the same reactive substance (to the precipitation test) may occur in very distantly related families, though they maintain that within such limitations its applicability is considerable.

Boyden (62) notes that there are two great principles which apply to the comparison of the relative intensities of serological reactions: "1. An antiserum will react more strongly with the particular antigen used in its formation (homologous reaction) than with equivalent amounts of other substances (heterologous reactions).   2. The intensity of reaction between an antiserum and various antigens is proportional to the chemical similarity of the antigens tested".   He points out that the results of serological tests are measures of the chemical similarity of the serologically active constituents of the sera of the animals compared and, therefore, literally indicate "blood-relationships".

Chester (90) furnishes a valuable critical summary of phytoserology to 1937, with a list of literature of 392 items.   He is decidedly favourable to the Königsberg (Mez) school in their claims as against the Berlin-Dahlem (Gilg and Schürhoff) school.   He claims that the great advantages of the method are its sensitivity, its specificity and its objective character.   He points out that the relationship reactions are "the summation of many or few identity reactions with the antigenic units in the protein complex of a plant extract".   The claim that the reactions are to the proteins of the chromatin would appear to be difficult to prove.

Boyden (61, 63), for animals, has strongly urged the value of serology.   He says: "In recent years evidence has accumulated that such parts of the biochemic constitution as are best revealed by the precipitin reaction appear to have unusual values in systematic zoology.   Thus, with an accurate and suitable method, the intensity of the interaction between antisera and antigens, such as serum proteins, parallels the systematic position of the species

where that is well known. The facts indicate that the serological natures of animals are such that serum proteins must be conservatively inherited and hence these data should rightly complement our morphological comparisons".

There is no need to enter here into various controversies that have arisen in connection with the application of serological data to phylogeny. Some of these are considered by Chester (90). Improved methods of technique have met some of these objections (60, 61, 90). On the other hand, a desire to construct a completed phylogenetic tree has outrun the validity of the data in such a diagram as the "Sero-diagnostischer (Königsberger) Stammbaum des Pflanzenreiches, 1924", in which even extinct groups known only from their fossil remains are given place! The following references will enable the student to trace most of the important literature on phytoserology: (16, 29, 31, 40, 48, 88, 89, 90, 160, 161, 249, 251, 267, 285, 302–305, 311, 313, 371, 481, 485, 490, 491, 503–505).

The choice of host plants by parasites is in part determined by the chemical constitution of the host. Thus, many animal parasites choose plants with similar chemical characters—mustard oil glucosides, HCN glucosides, alkaloids, organic acids (especially oxalic), salts, *etc.* In correlation we find insect larvae which, when free to choose, limit themselves to one plant family or to such families as have similar chemical substances. Thus, *Liriomyza cruciferarum* and *Ceuthorrhynchus contractus* generally live on the Cruciferae but are also found on related families and on Tropaeolaceae. *Pegomyia bicolor* occurs on Polygonaceae and Begoniaceae, families which are rich in oxalates, and *Agromyza reptans* on Urticaceae and Loasaceae with stinging hairs (72). The important problems of biologic races in different plant and animal groups have received considerable attention. The literature may be largely traced through the following papers and the references given in them: for bacteria (488), for fungi (66), for seed-bearing plants (450), for nematodes (168), for insects (435). It is clear from the examples and discussions in these papers that biological races play an important part in micro-evolution, and since they probably mostly have a distinct genetic basis, give first-class material for the action of natural selection, and strongly influence the ecological and geographical distribution of the organisms

concerned. Moreover, since they are amenable to experiment they should yield valuable data for the study of paramorphic phylogeny (paramorph = any taxonomic group within a species).

Palaeontological

As we have seen, it is usually not difficult to arrange living organisms in series in such a manner that differences in the structure of one or more organs appear as graded steps. Such series are frequently interpreted as phylogenetic or, if the study be concerned with only one organ, as morphogenetic or organogenetic. When the evidence is carefully stated and fully established, evolution may well be the most rational explanation of such series. The great diversity of opinion in published accounts of plant phylogeny suggests, however, that the available data are still too few for the construction of a valid general phylogenetic scheme—apart from difficulties inherent in the presentation of phylogenetic data and those inherent in the application of such data to classification. There is no doubt that the paucity of relevant palaeobotanical data in most groups of plants (partial exceptions are the Pteridophyta and Gymnospermae) is a major cause of uncertainty as to whether or not proposed series are phylogenetic, and, if they be, in which direction they should be read. Sufficient fossil evidence of the right kind gives a time sequence. To attempt phylogeny without it would be equivalent to an historian attempting to trace the historical development of, say, the British Constitution from its existing forms alone, from its complexities, traditions, absurdities, and, one must add, pragmatic strength, in the total absence of documents. The first thing in historical research is to obtain contemporary evidence. The first thing in phylogenetic research should be to obtain evidence from fossils. Palaeontological sequence alone does not, however, give a phylogenetic series, since, as Crow (115) has pointed out, mere sequence in the strata of successive geological periods does not prove or even necessarily indicate relationship. He adds: "in fact relationship of fossil organisms to one another must first be traced in exactly the same manner as that of living forms, and any arguments as to their relationships based on their stratigraphical arrangement are entirely secondary. They may confirm morphological data, but can never replace them. It is possible too that a living form may more closely represent the ancestral form of another liv-

ing species, or even of a fossil form, than any fossil species yet discovered". While this may be true, apart from some exaggeration in the use of the words "entirely secondary", it does not reduce the great importance of stratigraphical facts. A concise summary of the palaeontological evidence for evolution and examples of its use in constructing phylogenetic schemes is given in Newman (321). Davies (126) presents a clear picture of both the strength and the limitations of palaeontological data on the zoological side.

Since in certain groups of animals fossil evidence is relatively abundant and has been very carefully studied, it is useful to the botanist to know what general conclusions have been reached by palaeozoologists. Waddington (474) points out that there are two classes of evidence: "On the one hand, we can describe the general outline of the course of evolutionary change in a large number of organisms, in some cases even in a whole phylum. We are then dealing with phenomena which last throughout many geological periods, probably through times of the order of some millions of years. On the other hand, we have data as to the gradual alteration of one species or small group of species, a process which usually takes place within one of the more recent geological periods, over a period of a few million years. . . . On the large scale, whole orders and phyla seem to pass through an orderly series of changes which have been described as 'programme evolution'; while on the small scale it has been shown that a group of 'species' may evolve progressively along a certain line of change which has been called a 'trend'. 'Programme evolution' is particularly exemplified in the ammonites and graptolites. In the plant kingdom the nearest known approach is probably to be found in the ferns. In dealing with smaller scale evolution we meet with the concept of lineages".

It is not intended to give a full account of the uses and conceptions of the term lineage, as used by palaeontologists, since its use has been almost confined to palaeozoology; indeed, its application is possible only in such groups as can be studied as fossils in stratigraphically continuous series with abundance of specimens. Whole phyla of animals are completely excluded from its application because of the absence of such material, and there are no plant groups that have yet been studied with any approximation to the details recorded for such groups as ammonites and oysters. Nevertheless, the lineage concept is of very great importance in any general con-

sideration of phylogeny and taxonomy. A valuable, critical and thoughtful consideration of the problems involved is given by Swinnerton (420), and it will be of value here briefly to paraphrase and abstract some of his points. References to literature given at the end of Swinnerton's paper will enable the student to follow the subject further if he so desires.

Swinnerton notes that "in ordinary language the word 'lineage' is used in one or other of two senses. It is sometimes used in the narrow sense of our history books for the line of descent of more or less notable people and, along with other similar lines, is erected into a genealogical tree. In its broadest sense it relates to the descent of a whole race". Davies (126) defines a lineage as "a series of genera and species which form an evolutionary series, each one being ancestral to its successor in the geological sequence". The broader and narrower views are not always clearly differentiated. Lineages represented by palaeontologists as single lines, as in *Spirifer orestes* L., have been shown to have the structure of a "bifurcating branching system in which each section or line represents a separate line of descent". Trueman, after indicating the complexity of the internal constitution of a succession of communities, gentes or species groups, says: "In fact, the 'lineage' is rather a bundle of lines, or more correctly, a *plexus* of lines which repeatedly branch and reunite. In such a lineage there is a definite trend in the progressive characters, but also considerable variation in those characters at each horizon". Arkell (1935) says: "It is nearer the truth to say that the *Perisphinctes* of the Corallian form a plexus, the strands intimately linked and irretrievably tangled". After giving other similar examples of a band of anastomosing lines or a reticulation, Swinnerton rightly says that "for further light upon the structure of the plexus we must turn to the results of genetic experimentation". In free mating between bisexual organisms, and with the occasional appearance of mutations, a complex network can alone illustrate the distribution of characters from generation to generation.

On the other hand, "the recurrence of the same morphological species over a long range of time points to the constancy of the communal heritage for that period of time". Progenitors of the later members of a series of form species are to be sought not in the earlier members of that series but in the community as a whole to

which they belong. Successive mutations, in rates of growth, in unit characters or in size, may lead to a progressive development of some one feature which was already present in the ancestral community in an incipient condition. The total result is "a gradual change in the constitution of the plexus, which is reflected in a corresponding change in the character of the community as a whole, a character which is dependent not merely upon the range of variation of unit characters, or rates of growth in serial characters, but also upon the proportional distribution of these in the population as a whole".

It is clear that the more microscopically such a concept as lineage is examined, the more it is found to be a diagrammatic simplification of actual facts. To this extent it may be misleading unless the plexus of anastomoses be somehow indicated in place of lines. Trueman (446) has reached very similar conclusions from a study of certain lamellibranchs and gastropods in which he showed that there is considerable variation in any series collected at one horizon. "At each horizon variation appears to be continuous, and the characters, on the whole, vary independently. Each community is apparently homogeneous and indivisible. It is suggested that such evolving stock must be regarded as a plexus or bundle of anastomosing lineages".

Swinnerton (418) discusses the terms lineage, gens and plexus, which, with variable but not great differences in connotation, have been used to designate evolving community series of fossils. He attempts to link up, largely through the work of Huxley and Ford on growth rates in *Gammarus, etc.*, the findings of palaeontologists with the results of genetical experiments. Bather (33) stated that "the whole of our system, from the great phyla to the very unit cells, is riddled through and through with polyphyly and convergence". He concluded that "important though phylogeny is as a subject of study, it is not necessarily the most suitable basis of classification. For three reasons: the first, that the more complete the phylogenetic tree, the further must it depart from a classification based originally on different principles; the second, that the more refined our analysis becomes, the greater is the difficulty of representing its results in any classificatory scheme at all; the third, that although the source of the raw material may, philosophically considered, be a more important field of enquiry than the processes by

which it is elaborated into the varied forms we know, still a classification which obscures the qualities of the goods as delivered loses thereby in practical value". Calman (75) considers Bather has exaggerated the all-pervading occurrence of convergence; nevertheless, he himself gives striking examples of it. He contends that a natural system does exist, and for it "no scientific explanation other than that offered by community of descent has ever been given".

Woodward (500) has pointed out that our increasing utilization in classification of "extinct animals makes the use of the Linnean method of classification very difficult. . . . We can only make satisfactory classifications when fossils enable us to trace the ancestors of the various groups through a series of geological epochs. Instead of classes, orders, families, and genera, we are concerned with phyla, lineages, branches, and grades; and we soon discover that related lineages pass through the same orderly succession of changes by which the same—or nearly the same—result is eventually attained. This means that when we are dealing only with existing animals or the associated animals of any single geological period, the families and genera, at least, which we establish consist of members that are not so closely related as they seem to be. They are merely the end shoots of a series of parallel lineages which have reached the same grade of development". He cites striking examples of parallel development in the lineages of various groups of animals, invertebrate and vertebrate and even among the highest vertebrates.

An example of a concentrated effort to trace the evolution of a definite group, mainly by the use of palaeontological and distributional data, is provided by Macfarlane's work on the fishes (280).

Arkell and Moy-Thomas (236) have pointed out that "palaeontological classification, like all classification, must primarily be useful. It should provide a practical means by which fossils can be identified and compared". Classification has, however, come to have the dual aim of providing an easy means of recognizing fossils and of giving a summary of existing knowledge of phylogeny. "It is part of the palaeontologist's business to unravel phylogeny by the study of fossils; but he can only arrive at an approximation to the truth, for he has only a fraction of the material to work on, the hard parts which alone have been preserved by fossilization. At the best of times his results are hypothetical and more or less subjec-

tive, and it may be questioned whether such results form a suitable foundation on which to build a classification and a nomenclature. . . . The major problems of palaeontological taxonomy may be summarized by saying that a phylogenetic and a practical classification are frequently incompatible, and that lack of uniformity in scale and unity of purpose introduce further confusions". An interesting difficulty is illustrated by the ammonites: "a number of lineages of ammonoidea can be traced up through the Devonian rocks, all undergoing more or less parallel evolution. They may be classified in two ways: either 'vertically', making the genera correspond with the lineages, along which the successive forms are marked by parallel species, or 'horizontally', making the genera correspond with successive grades irrespective of the lineages". The chief objection to the 'vertical' system is the uncertainty of recognizing true lineages. "Since palaeontological classification is intended to be practical, no useful purpose is served by complicating it with detailed phylogenies, which are often of little interest save for demonstrating convergence". The authors make a strong plea to palaeontologists to avoid upsetting established nomenclature.

Returning to plants, we find that fossil evidence is meagre, except in the Pteridophyta and Gymnospermae, in so far as it is relevant to phylogenetic problems. In the realm of micro-evolution we have no evidence of lineages at all comparable to that in such animal groups as the graptolites, ammonites or oysters, or even such relatively continuous series as those for the horses or elephants. The nearest approach to a plant lineage is found in the work of Chandler (86) on *Stratiotes*. This, however, is based only on a series of fruits, and the characters employed are thus limited to one organ. On a larger evolutionary scale the series of fossil ferns and especially their stem anatomy may be mentioned. Again, it seems certain that the Gymnospermae preceded the Angiospermae very considerably, even on a geological time-scale. It is, therefore, to this extent palaeobotanically sound to suppose that the latter may have arisen from some group or groups of the former.

It is, perhaps, of considerable significance that some of those who have specialized in palaeobotany are most critical of its value in reconstructing phylogenies with the data available at present. Thus, Scott (386) says: "Like Dr. Lotsy, I have become sceptical of late as to most phylogenetic reconstructions, but one need not go

so far as he does; in 'dim outline', at all events, we may still hope to catch glimpses of the course of evolution". Thomas (433, 434) concludes that what palaeobotanical evidence is available is completely at variance with the more or less widely accepted views as to the origin of the Angiospermae. Excellent general summaries, with numerous references and judicious conclusions and inconclusions, of what is known regarding the history of plants are provided by Seward (390) and by Darrah (124).

The origin of the Angiospermae remains a matter of speculation in the absence of any fossils connecting them with another group or with other groups. The following palaeobotanical facts indicate the main available data under our present heading. The oldest known fossil evidence for Angiospermae is in the form of pollen from Jurassic rocks, (394, 395) determined as belonging to Nymphaeaceae, Magnoliaceae and (?) Juglandaceae. No other remains of Angiospermae have been recorded from rocks older than the Lower Cretaceous. The Caytoniales (428, 201) were apparently, at least in part, angiospermous, but they can not be classed with any of the modern Angiospermae. Stopes (413) has described petrifactions from England of stems of Aptian (Lower Greensand) age: *Aptiana radiata,* "in every detail like one or other of the modern, highly specialized types of Angiosperms"; *Woburnia porosa,* the structure "points to a Dipterocarpacean affinity"; and *Sabulia Scottii,* with wood characteristic of the higher dicotyledons. Other genera of dicotyledons of Aptian age, but of uncertain affinities, have also been described by Stopes (414). Seward (389) was not able exactly to correlate the Cretaceous series in Greenland with those of western Europe, but the fossil flora agrees most closely with the Lower Cretaceous floras of Europe and North America and with floras of the Old and New Worlds which are usually regarded as Cenomanian in age, and is probably to be regarded as equivalent to Neocomian-Cenomanian or -Turonian. He recognizes the following families as occurring in these rocks: Palmae, Liliaceae (?), Fagaceae, Moraceae, Menispermaceae, Magnoliaceae, Lauraceae, Platanaceae and Leguminosae. Stopes and Fuji (415), from Cretaceous rocks of Hokkaido, Japan, describe a monocotyledon and five dicotyledons (four Monochlamydeae and one Dialypetalae). The specimens are placed in or near Saururaceae, *Juglans, Populus, Fagus, Sabia* and Liliaceae. The published

accounts of the Cretaceous floras of Portugal, North America and other parts of the world are too numerous to summarize here. While the taxonomist may doubt some of the identifications, they tell a story essentially similar to those recorded above: that by Cretaceous times numerous families of monocotyledons and dicotyledons were already in existence. Archichlamydeae of a woody habit predominate as determinable fossils. This may be an accident of preservation, and the negative evidence of scarcity of herbaceous types must not be stressed. There remain important facts of the absence of any plant recognizably more primitive than known existing families and the relative wealth of morphological structure (vegetative, floral, fruit and seed) of the families represented in Cretaceous, and even Lower Cretaceous, strata. It would appear either that the main development of the angiosperms was long pre-Cretaceous or that their diversification at about the family level took place almost immediately and suddenly after their first appearance and possibly as a result of their mode of origin.

The researches of C. and E. M. Reid, Chandler, Bandulska and others throw much light on the history of Cainozoid floras, but their value for taxonomy and phylogeny concerns details rather than general schemes and principles or origins of major groups.

Darrah (124), after summarizing the palaeobotanical evidence available for a study of the phylogeny of the plant kingdom, lists, on a morphological basis, a number of "fundamental inventions or developments in the history of the tracheophytes" (vascular plants). This list is well worth quoting, since it epitomizes the more important of the generalized problems which have interested palaeobotanists and others in recent years:

"1. The invasion of land by an undifferentiated thalloid plant (not later than Silurian).

"2. The simple upright undifferentiated and protostelic axis (apparently Silurian).

"3. Enlargement of the plant body with specializations towards a division of labor—sterile and fertile (apparently late Silurian).

"4. Origin of the photosynthetic leaf (Devonian).

"5. Specializations in the service of support, with the resultant secondary body (Devonian).

"6. Development of the strobilus (Devonian).

"7. The development of heterospory (upper Devonian if not earlier).

"8. Retention of the gametophyte within the megaspore, within the megasporangium, where fertilization takes place, with the ultimate attainment of (a difference only in degree) the seed (probably upper Devonian).

"9. The evolution of the pollen-grain.   The same tendency as the retention of the megagametophyte (Carboniferous, if not earlier).

"10. Evolution of the bisporangiate flower (Triassic).

"11. Attainment of the condition of angiospermy (Jurassic or Triassic).

"12. Evolution of the herbaceous habit (Cretaceous, but chiefly Cenozoic)".

Cytological

Cytology is now-a-days regarded very largely as the study of the mechanism of heredity and variation.   That it is already extending into biochemistry and biophysics is merely another example of breakdown in the artificial stratification of science, indeed, of knowledge as a whole.   To say that cell, and particularly chromosome, structure and behaviour are essential in the study of heredity and evolution and therefore in the study of taxonomy and phylogeny, is in no way to detract from the importance of other lines of research bearing on the same subjects.   Much nonsense has been written regarding the supposed exclusive or predominant value of single viewpoints, in both science and philosophy.   Cytology is no more ultimate than, say, taxonomy or ecology.   Nevertheless, cytology has a present prominent position in biology, firstly because of great recent advances in our knowledge, particularly of karyology, and secondly because it has become closely linked with genetics, whence the use of the term cytogenetics.   Schurhoff (384), Darlington (121), Sharp (392) and White (487) have, amongst others, given us learned text-books which are not always written so that a non-specialist can understand them.   The writer believes that cytologists and taxonomists must co-operate with reciprocal advantage in the future much more than they have in the past if the maximum possible advance is to be made in biology.   Some of the more general relationships which must be developed between taxonomists and cytologists have been stated by Turrill (456). Here we must be limited to a few references to recent studies and aspects selected from the enormous number available.

The number, structure and behaviour of chromosomes give characters which, in general, are constant or referable to a constant common plan for a species or other taxonomic group. When used in classification, other than a special classification, such characters must be evaluated with other characters, morphological, genetical, *etc.* Anderson (7) has reviewed the relationships between cytology and taxonomy. Smith's work on *Anchusa* (404) is an excellent illustration of the reciprocal support given to conclusions reached by taxonomic and cytological research, respectively. Cytological data have, at present, special taxonomic and phylogenetic value at or about the species level in determining origin by polyploidy, hybridization, *etc.* Thus, the hybrid origin of *Spartina Townsendii* (228), *Galeopsis Tetrahit* (315, 316) and certain species of *Rubus* (367), *Antirrhinum* (35) and *Salix* (324, 325, 326, 327) has been cytologically and/or genetically established, at least in so far as a species is determined by morphological and cytogenetical characters. In certain genera, and larger groups too, the cytology of large numbers of species is so uniform as to be of little or no help in taxonomic differentiation. This is true, for example, in the genus *Rhododendron* (378). At the other extreme in the genus *Carex* (475, 209, 210) the species show little or no karyological resemblance from one to another, at least in chromosome number. Wahl (475) found "The haploid numbers form an aneuploid series ranging from 13 to 56. A comparison of all the known chromosome numbers in the genus indicates the aneuploid series may be secondarily derived from euploid series with different basic numbers, of which 7 is probably most often represented". The taxonomy and cytology of *Primula* have been correlated, as far as possible, in the work of Bruun (71) and Wright Smith (405). To a great extent cytological data confirm the taxonomic results in this genus. Average size of the chromosomes was found to be the most constant character, and mere numbers may be misleading for phyletic purposes. The broad cytological data have more value as sectional characters than for the alignment of closely related species. The cytological work on *Rosa* by Tackholm (421) and others, on *Triticum* as summarized by Watkins (479), on Cruciferae by Manton (287) and on *Crepis* by Babcock and others (26, 27) further serves to illustrate the increasing linkages between taxonomy and cytology.

Connections between cytological research, phytogeography, ecology and taxonomy at or below the species level are well illustrated by Manton's work on *Biscutella* (288) and Hagerup's (182, 183, 186), Tischler's (440, 441, 442), Rohweder's (363) and Fischer and Schwanitz's (144) studies on polyploidy in various floras and ecological groups.   Bowden's researches (52), however, in contrast to those of Hagerup and Tischler and his colleagues, "do not support the theory that polyploids are usually hardier than diploids and are therefore better adapted to climatically unfavourable regions".   Stebbins (411) concluded for dicotyledons that "Gross changes in the chromosomes have been more significant in producing new types of growth habit than in differentiating new families and orders".

Difficulties are bound to arise in attempts to co-ordinate results in two disciplines so different from one another as cytology and orthodox taxonomy.   Thus, the taxonomist has perforce to take more criteria into account than the cytogeneticist in his delimitation of species.   The taxonomic status of polyploids and of apomicts awaits stabilization, though, perhaps, this should not be attempted in any rigid manner till further data are available from a wide range of lines of research.   Definitions of such oft-used terms as hybrid, affinity and environment frequently differ from author to author and lead to ambiguities.   Other difficulties are inherent in the material. Darlington (122) gives examples showing that genetic systems can change while external forms remain the same.   The cytologist has thus to make distinctions which are entirely beyond the scope of the taxonomist.   Fairly considerable numbers of taxonomic species have different chromosome numbers in different individuals (408). The increasing number of known examples of plastid and cytoplasmic mechanisms influencing inheritance adds complications which will afford problems to both the taxonomist and the cytologist.

As contrasted with genetics, it might be expected that cytology would give help in elucidating problems of taxonomy and phylogeny much above the species level.   Sufficient cytological data are, however, available at present in only a few groups for detecting possible phylogenies above the genus level.   Such data are increasing rapidly, and a valid general survey for the angiosperms may not have to wait many decades.   The great wealth of tropical floras has, however, been scarcely touched cytologically, and till investigations on

a fair proportion of tropical species have been made, general conclusions may be dangerous because they would depend on data with a phytogeographical bias. The uniformity of gymnosperm cytology (377) and the cytological relationship of the Pomoideae to the rest of the Rosaceae, with a probable hybrid origin of the whole group followed by secondary polyploidy (123, 307, 379), are examples of facts and conclusions of use in taxonomy and phylogeny which may be expected in increasing number and scope. Anderson (4) has suggested from cytological data that the Magnoliales may have originated from wide crosses between different groups of Gymnosperms.

Genetical

Genetics, essentially an experimental subject, has its greatest importance for taxonomy and phylogeny at about the species level. Turrill (456) gives records of a number of wide crosses and suggests that wide crosses on a large scale might be attempted under diverse conditions. That an F_1 might arise and by chromosome doubling give rise to a fertile allotetraploid, even from parents taxonomically very diverse, is always a possibility that might give startlingly interesting results.

The continued distinctness of groups designated as species by the taxonomist depends on intraspecific inbreeding and interspecific isolation. There are many kinds, causes and degrees of isolation resulting in lack of uniformity in the taxonomic concept of plant species. The zoologists have, in theory but not always in practice, emphasized intersterility of groups as the most important specific criterion. Cytogeneticists tend to follow this zoological lead. The botanist knows that for plants such a criterion used primarily would lead to a special classification of little or no general use. This, of course, in no way detracts from the importance of genetically controlled sterility as a cause of isolation of many species, one from another. Such sterility may be absolute and must in time be followed by divergence or increased divergence. An excellent example within one genus and a limited geographical area of the taxonomic importance of sterility due to cytogenetic causes is furnished by the British knapweeds (290, 293). *Centaurea nigra, C. nemoralis* and *C. Jacea* hybridize freely wherever they grow together, producing complex hybrid swarms, some of whose members have been described as distinct species. *C. Sca-*

biosa is frequently found growing with one or more of these species and often in the midst of the hybrid swarms, but it always remains distinct, and no hybrids have been observed by the authors in very extensive and intensive field investigations or made by them in reciprocal matings in controlled experiments.

Danser (119, 120) has proposed a taxonomic scheme, with the introduction of new categorical terms, based essentially on sterility-fertility criteria. A *comparium* is the sum total of individuals which can be combined directly or indirectly by crossing; a *commiscuum* is the sum total of individuals which can be combined directly or indirectly by mixture, *i.e.,* by crossing, which results in the production of fertile hybrids; a *convivium* is, within a commiscuum, a group of individuals which can be distinguished from other groups by more or less sharp characters and which is maintained in some degree of isolation by conditions other than cross sterility (ecological, phytogeographical). Turrill (456) has criticized this scheme from the standpoint of its possibly replacing the normal taxonomic procedure. Danser's ideas, however, will have to be taken into consideration when the time comes for the construction of a more generally useful taxonomy than has yet been published.

Clausen (102), after quoting a series of examples, has well summed up the value of crossing as a criterion of phylogeny as follows: "Crossing possibilities do not necessarily imply phylogenetic relationship but probably only a certain kind of genic affinity; the morphological relationship dealt with in taxonomy is probably for the main part contingent upon genic conformity, not always on a joint phylogenic development". Sax (381) also shows that it is wrong to assume that the degree of chromosome pairing in species or generic hybrids "can be used as an index of chromosome homology and species relationships".

Anderson (8, 9) has shown that there are at least four hindrances to character combinations in F_2 populations from crosses. These are imposed by gametic elimination, zygotic elimination, pleiotropism (especially spurious pleiotropism) and linkage. Since there is increasing evidence that hybridization has played a very important part in the evolution of plants, and consequently must be considered in phylogenetic theories and schemes, limitations to its effects and any means by which discontinuities can be pro-

duced in hybrid populations must concern the taxonomist and the phylogenist.

Correlation of characters used in taxonomy is given considerable weight both in classification and in phylogenetic theory. Cytogenetics can establish the degree and sometimes the cause of such correlation. For the gall-wasps (*Cynips*), Kinsey (245, 246) has made an attempt to correlate a taxonomic study with the data of genetics. He believes "species to be realities which are large populations representing interbreeding Mendelian races having access to a common stock of genes". Further, "It has been one of the major contributions of genetics to show that similarity is not wholly safe as a criterion for its recognition of relationship. . . . It is only when phylogenetic interpretations are based upon a variety of morphologic, physiologic and distributional data that true relationship in such instances becomes evident."

There appears to be no generally applicable genetic criteria by means of which alone characters can be evaluated as of varietal, specific or generic value. In *Silene*, Marsden-Jones and Turrill (289), and in *Malva*, Kristofferson (250) have definitely reached this conclusion. Matsuura (296) quotes numerous crosses between two distinct taxonomic species which behave genetically in the same manner as varietal crosses. At the same time, as Matsuura says, genetic "analysis of the specific characters offers biologists a basis for a much clearer understanding of the fundamental constitution of the species and of the nature of the limits of taxonomic groups". In *Viola* and in *Gossypium* interspecific crosses reveal some minor differences in genetical behaviour from that in intraspecific ones. Thus, Harland (197, 198, 199, 200) explains that in certain cottons varietal crosses show segregation of modifiers which may partially or entirely obscure the distinction between dominant and recessive characters.

Anderson (6) raises a number of important questions on this subject without supplying answers to them. He does, however, make the statement "that changes in emphasis are of specific rank, while differentiation separates categories higher than species". Epling (141*a*) points out that it is difficult to distinguish between changes of emphasis and differentiation, and finds, contrary to Anderson for *Aquilegia, Narcissus, etc.*, that "in Labiatae the categories higher than species are characterized, just as the species

themselves, not by a change in pattern necessarily, but by different combinations of changes in emphasis. Such combinations in reality produce new patterns".

Blakeslee, Murray and Satina (51) show that in the genus *Datura* crossability fails to run parallel with taxonomic relationships, as established by sections in the genus, and crossability may be different in reciprocal matings.

Dobzhansky (132) deals with many of the problems in which genetics throws light on the origin and nature of species. It is, however, doubtful whether some of his conclusions can apply so widely in the plant kingdom as to make them valid biologically. This is particularly true of his conception of a species. What the plant taxonomist considers with full justification as good species—what for general botanical purposes he must accept as species—are often not nearly such discrete units genetically or cytogenetically as postulated by Dobzhansky and other zoologists for animals. The view that species must be physiologically incapable of breeding one with another to produce fertile offspring can not be maintained in practice in plants to give a generally useful classification. It could be maintained consistently to give a special classification, possibly with interesting results, but these would be of limited use.

Parallel variations are known in many groups of plants and animals and led Vaviloff (462) to enunciate his well known "law of homologous series in variation". Matsuura (296) has, however, pointed out that up to 1935 for no group has "the genotypic identity of parallel variations in different plant species been demonstrated in the exact sense". He shows that what he calls "the method of diallel crossing" is the only way with certainty "to disclose genotypic differences, if any, between the parental species, even though they are phenotypically alike", and "at the same time to discover genotypic identity between them even though they are phenotypically dissimilar". The method consists in comparing the results from all possible combinations within and between two species for the pairs of dominant-recessive characters studied. He gives, however, numerous examples of probable genetic parallelisms within a genus and within a family and concludes that a high degree of germinal composition and organization in common may be deduced on the occurrence of genotypic parallel variations. Further, he

argues that taxonomic categories are only conventional and all characters, specific, generic, or family, "compose each and every species, and all should be alike in their genetical behaviors. The distinction between them is merely dependent upon the degree or extent of characters which the organisms have in common".

As with cytology, so with genetics, the genetical species or other group does not necessarily coincide with the taxonomic. Raunkiaer (350) implies this when he points out that genetics evaluates the individual by its offspring, systematics by what it is itself. With the necessary extensions the idea is applicable to all groups. Systematic units rest not on the genealogical principle but on the similarity principle. Phylogeny is not absolutely necessary for systematics, but systematics is for phylogeny. This is no doubt true, but one can add that genetics, or better, cytogenetics, is also essential for a proper understanding of phylogeny and for the establishment of valid phylogenetic schemes.

The relationship between ordinary taxonomy and experimental taxonomy has been discussed recently by Turrill (236, 454). The valuable researches by Clausen and his colleagues in America (106) also deserve the close attention of taxonomists and phylogenists. There is no doubt that experiments are essential before the taxonomy of many groups at or about the species level can be made most useful by being developed on a sound basis and before their probable phylogeny can be profitably discussed. A knowledge of genetic relationships is more basic than a knowledge of phylogenetic relationships. Genes may be aggregated in one individual or taxonomic group from many ultimate sources which can be traced only after prolonged investigation and detailed genetical analysis.

The taxonomic value of genetics is so great, its methods and outlook are of such educative value to the taxonomist, that it would be advantageous for every taxonomist to have a training in genetics. The converse is also true and every geneticist should have a training in taxonomy. Turrill (455, 458) outlines desirable relationships between taxonomy and cytogenetics with suggestions of problems needing co-operative research. Clausen (103) also has correctly urged the need for closer co-operation between genetics, taxonomy and field-studies. Thus, he says: "As long as genetics is concerned with the individual genes only, it makes no difference

which type of material [wild or cultivated] is utilized. But genetics of our days is going further, trying to apply its findings toward a revision of our conception of living organisms, their diversity and mutual relation as well as the origin of this diversity. This demands a closer contact with the living organisms in natural surroundings. Only to a very limited degree have geneticists studied the genetic interrelations of taxonomic units that are the immediate products of evolution as they invitingly offer themselves under such conditions". Clausen's own work with *Viola* (100, 101, 102, 103, 104, 105) and Marsden-Jones and Turrill's work with *Silene* (289), *Centaurea* and other plants (290) illustrate the new line of attacking micro-evolutionary and micro-phylogenetic problems. Marsden-Jones, Summerhayes and Turrill (294) discuss the need for the introduction of certain taxonomic methods into cytogenetical and ecological research. They ask especially for greater care in specific and varietal determinations and for the preservation of samples of the materials studied.

The tremendous practical use of combined taxonomic-genetical-ecological studies can scarcely be better illustrated than by the recent work on malaria-carrying mosquitoes. *Anopheles maculipennis* has been shown to be not a homogeneous species but a group of at least six widespread varieties which morphologically differ in little but egg type. In physiological characters there are profound differences between them in mode of hibernation, sexual behaviour and even host preferences. Preliminary breeding work showed sterility, complete or partial, between one of the varieties, *atroparvus,* and the other five, *messeae, elutus, typicus, melanoon, labrachiae*. Other breeding experiments were unsuccessful. Two or more "varieties" often occur together in the same area. It would appear that sterility mutations have preceded morphological differentiation and geographical isolation. The existing diagnosis and classification of species in *Anopheles* by taxonomists is inadequate, and, as Hackett (180) says, "The epidemiologist therefore is inclined to follow the geneticist, for to him behaviour is everything and morphology is only a tool".

Ontogenetical

Recapitulation, the dictum that ontogeny repeats phylogeny, has been much used by zoologists. A useful concise summary of re-

capitulation, followed by a critique, will be found in Newman (321). The supposedly simple and straightforward comparisons made by the earlier phylogenists between phylogeny and ontogeny have not, on the whole, stood the test of more recent research. Such research, with references, has been ably summarized by de Beer in a number of easily accessible books (36, 37, 236), and it is not necessary to give details here. Nevertheless, some of his conclusions have a wide biological applicability, and quotation of some of them may direct the attention of botanists to a branch of study which has been somewhat neglected on the plant side in recent years.

De Beer reaches the following conclusions relevant to our subject: "Ontogeny is the result of the action of external factors in evoking responses from the internal factors of an animal to which the latter were transmitted by inheritance from its parents.

Phylogeny is provisionally to be regarded as a series of adult forms, which are disconnected and causally unrelated to one another, each adult form being the result of an ontogeny which differs from the previous one.

Successive ontogenies are related to one another by the transmission of internal factors from fertilized egg to fertilized egg.

Modifications in ontogeny (in a constant environment) are due to changes in the internal factors.

Phylogeny is therefore due to modified ontogeny.

Phylogeny plays no causal part in determining ontogeny.

The internal factors exert their effects at certain definite rates.

Modification of the rate of action of the internal factors in successive ontogenies will result in heterochrony [alteration and reversal of the sequence of stages as shown in ontogeny compared with phylogeny].

Evolution is brought about by acquisition of qualitative novelities, and by the production of novel situations by quantitative alteration of the rate of action of the internal factors.

New characters may appear at all stages of ontogeny, and by heterochrony they may be retarded or accelerated, so as to appear later or earlier in subsequent ontogenies.

Characters present in the early stages of ontogeny have (provided they are not too specialized) played an important part in evolution by paedomorphosis [the introduction of youthful charac-

ters into the line of adults], resulting in large structural changes without loss of plasticity.

Characters present in the late stages of ontogeny have played an important part in evolution by gerontomorphosis [the production of phylogenetic effects by modifying characters which were already present in the line of adults], resulting in relatively small structural change with loss of plasticity.

Paedomorphosis and gerontomorphosis may act alternately in the phylogeny of a race, the former producing racial rejuvenescence, the latter racial senescence.

Recapitulation, *i.e.,* the pressing back of adult ancestral stages into early stages of development of descendants, does not take place, although there may be acceleration of one structure or another.

Repetition of ancestral ontogenetic stages in the ontogeny of the descendant, whether retarded or accelerated, is due to the transmission of internal factors from ancestor to descendant.

Similarity in ontogeny between any animals is proof of their affinity, and no evidence as to the adult structure of the ancestor.

Atavism is due to the re-establishment in the ontogeny of the descendant of a set of circumstances which was present in the ontogeny of the ancestor. . . . phylogeny does not explain ontogeny at all. . . . For past phylogeny no method of study other than the historical descriptive is possible. But since phylogeny is but the result of modified ontogeny, there is the possibility of a causal analytic study of present evolution in an experimental study of the variability and genetics of ontogenetic processes".

Recapitulation as a guide to animal phylogeny, though heavily criticized by some embryologists, is much used by palaeontologists. Lang (260) has said: "While embryologists may admit recapitulation, palaeontologists use it as a guiding principle". The evidence for a "reinstatement" of recapitulation "in its proper place as an established principle of evolutionary thought" is given by Swinnerton (419).

Turning now to the botanical use of ontogeny in its relation to the determination of phylogeny and affinities, we find that it has been, on the whole, much less stressed by botanists than by zoologists. In some groups of the vascular cryptogams the ontogeny of the stelar structure of the stems has been frequently accepted as indicating phylogeny or, perhaps it should now be said, organo-

genesis. A useful summary of the facts interpreted by one or more botanists as supporting the theory of recapitulation is provided by Sahni (369) for vascular plants—mainly vascular cryptogams and gymnosperms. In the earlier part of this century a great deal of laborious research was devoted to the structure of the seedlings of phanerogams, particularly to the anatomy of their hypocotyls and the details of transition from root to stem. The following are references to a selection of these papers and through these others can be easily traced: (216, 217, 218, 219, 220, 426, 427, 265, 108, 109, 422, 215, 148, 224, 372, 373, 375, 445, 59). The results were not commensurate with the time and trouble involved. This does not mean that the structural details discovered will not one day be fitted into a wider scheme, but, as shown for example by Hill and de Fraine (218, 219), the enormous importance of physiology in questions relating to vascular tissues may so modify their structure as to outweight their value as indicators of phylogeny, which was in the main the background against which the researches were undertaken. A summary of recent work on the application of causal principles to seedling structure is provided by Thoday (425).

The numerous examples of juvenile structures which apparently resemble adult organs in supposed allied or ancestral species or genera are too well known to need giving here. It may, however, be suggested that a re-investigation of these on a wider and more systematic comparative basis is desirable. It might well be possible to bring the facts into line with the modern findings on the zoological side which are outlined above.

Gundersen (174) gives examples from the ontogeny of the angiospermous flower which appear to support widely accepted views of the relative age of certain groups of angiosperms, especially dicotyledons, in so far as these are based on floral structure and presumed organogenesis. He points out that the following tendencies are widely accepted as progressive in organogenesis and agree with the findings in development:

> Petals: from separate to united.
> Petals: from actinomorphy to zygomorphy.
> Sepals: from separate to united.
> Ovary: from superior to inferior.
> Carpels: from separate to united.
> Placentation: from parietal to axile.

Ecological and Phytogeographical

Ecology resembles genetics, at the present stage of development of these two branches of biology, in that they both depend on taxonomy to give them their "degrees of latitude and longitude" in what would otherwise be a chaos of animal and plant individuals, that both work mainly at and below the species level, and that, consequently, neither can yet give much help in tracing the phylogeny of higher taxonomic categories. Since, however, species problems are the centre of modern dynamic biology, the taxonomist must expect to meet ecological problems in his research. Salisbury (in 236) has recently outlined the reciprocal relationships of ecology and taxonomy. Like Turrill (in 236), he calls attention to the importance of plasticity and genetic variability in both taxonomic and ecological studies. The selective value of relatively constant genetic variations has been much studied, but the selective value of plasticity itself, especially in allowing a wide or extending ecological and geographical distribution, has not received much attention of late from botanists, and the subject is recommended for investigation by modern methods. The occurrence of pairs of species which are kept distinct only because of ecological barriers is well illustrated by field and breeding studies on *Silene* (289, 453). Salisbury (in 236) and Turrill (457) give many other examples which have not, however, been worked out in so much detail. Since differentiating groups of organisms are constantly being subjected to the sieve of natural selection, we must expect the climatic, edaphic, biotic and historical factors on an ecological time and space scale to be important links in the causal chain leading to divergence in micro-evolution and, usually in a more obscure manner, on a phytogeographical time and space scale, in taxonomically higher grade evolution. It may be suggested that differences in ecological behaviour between closely similar species should yield excellent material for a study of the way in which the genotype, physiologically, biochemically and biophysically, controls the appearance of phenotypic characters.

Haldane (187) has suggested that very high mutation rates due to heat may have played a part in evolution. It may have caused orthogenetic evolution of species near the tropical limit of their range, and thus may be partly responsible for the greater diversity of species found in tropical as compared with temperate and arctic

habitats. Hagerup (182, 183, 186), Tischler (440, 441, 442, 443) and others (363, 144, 423, 370, 297, 461) have produced evidence which appears to indicate that polyploidy is greater under more extreme or peculiar ecological or phytogeographical conditions.

Character-gradients within groups have been termed *clines* by Huxley (233, 234, 236). The observed gradient in visible characters can frequently be shown to be correlated with some climatic or edaphic gradient. Clines of one sort or another are probably of frequent occurrence in nature, and the same idea can be applied with advantage to widely cultivated plant groups. Many of the distributions for cereals, legumes, *etc.,* worked out by Vaviloff (463, 464, 465), appear to be examples of clines. Turrill (452) describes the series of morphological changes in *Ajuga chia—A. Chamaepitys* which can be traced in populations from the Levant and Aegean areas across Europe to the British Isles. These constitute a gross cline.

In the genus *Leontopodium,* Handel-Mazzetti (196) regards adaptations to extreme ecological conditions as indicating non-primitive species.

Taxonomists have increasingly recognized the importance of geographical distribution as an aid in both determination and classification. The value of phytogeography as a subject in its own right became obvious after the classical studies of J. D. Hooker, Asa Gray and Engler. The literature of plant distribution is now enormous and a modern synthesis is greatly needed. The attention of botanists may also be called to the works of Rensch (358) and Reinig (356). Here only one or two items of immediate relevance can be discussed and these only briefly.

Geographical isolation and the controlling influence of barriers are widely, and sometimes not very critically, accepted as main causes in keeping taxonomic units and communities of vegetation distinct. Seas, mountain-ranges, deserts and other physiographical obstacles to extension of range of plants and animals were particularly mentioned by Darwin (125) in his account of geographical distribution, lending support to the theory of natural selection. Much research has been devoted to this subject at about the species level. The work of Gulick on the land snails of the Sandwich Islands (172, 173) is worth the consideration of botanists. Many

modern investigations concerning the importance in micro-evolution of physical factors are referred to in greater or less detail by Huxley (235) and by various authors in "The New Systematics" (236). Geographical isolation is frequently accompanied by genetical isolation and it is often impossible to say which arose first. Not infrequently, however, widely discontinuous geographical ranges are found in species which hybridize when they are artificially juxtaposed, as in gardens. A good example is furnished by the London plane. Concerning this Sax (380) says: *"Platanus acerifolia,* a natural hybrid between *P. orientalis* of south-eastern Europe [and N. Asia Minor] and *P. occidentalis* of North America, has 21 pairs of chromosomes which pair regularly at meiosis. The hybrid is fertile, and genetic segregation occurs in the second generation. These facts indicate that the parental genoms are similar and are compatible, even though the parental species have been isolated for a long period of time". A chapter is devoted by Robson (361) to isolation as a factor in the divergence of species. Rothschild (366) gives a number of excellent illustrations from insects of the interrelations of systematics, phylogenetic research and geographical distribution.

Wettstein (483), after consideration of the dual purpose of systematic botany to trace and express phylogenetic connections and to provide "eine möglichst klare und eine rasche Orientierung zulassende Uebersicht über die bisher bekannt gewordenen Pflanzen zu geben", says: "dass die entwicklungsgeschichtlichen Beziehungen der Pflanzen zu einander so mannigfach und so vielfach durchkreuzend sind, dass sie eine Darstellung in Form eines logisch aufgebauten Systems nicht zulassen". The following very balanced conclusion is reached: "Ein vollständiger Aufbau des Systems auf phylogenetischer Grundlage wird daher kaum zu erreichen sein, wir müssen damit zufrieden sein, wenn das System soweit als möglich ein Spiegelbild unserer phylogenetischen Kenntnisse abgiebt und müssen mit der eventuellen Notwendigkeit rechnen, phylogenetische Erfahrungen in Form des Systemes selbst nicht zum Ausdrucke zu bringen". He also makes the very wise suggestion (he terms it a necessary concession) "dass descendenztheoretische Erfahrungen nur dann in der Systematik Verwertung finden sollen, wenn sie als hinlänglich begründet angesehen werden können. Bevor dies der Fall ist, diese Verwertung vornehmen, heisst in ganz überflüssiger Weise die Systematik zu einer swank-

enden und die Zwecke der Orientierung ganz ausser acht lassenden machen".

Wettstein points out that for the largest groups it is easy to make a sequence which, on certain reasonable assumptions, may be considered phylogenetic, as: heterosporous pteridophytes——gymnosperms——angiosperms. The smaller the groups considered, however, the greater become the difficulties. These are exceedingly great at the generic level, and "Vollständig unmöglich aber wird eine derartige Anwendung der Phylogenie zumeist in der Systematik der Arten". Especially because of frequent morphological convergence, the morphological method alone can not lead to a sound phylogeny. On the other hand, he believes that a combination of detailed morphological and phytogeographical (in the broad sense) studies shows that "Pflanzenarten von weiterer Verwandtschaft in Arealen vorkommen, die durch mehr oder minder grosse Gebiete getrennt sind oder aber in denselben Gebieten leben können; sei weichen naturgemäss durch grössere morphologische Unterschiede voneinander ab und sind im allgemeinen nicht durch nicht-hybride Uebergänge miteinander verbunden". He considers that this dual method of comparative morphology and distribution is an objective means of determining relationship at and about the species level. In an area, such as much of Europe, where the post-Tertiary geological history is well known, the method can be used advantageously with the data derived from a study of the influence of the Quaternary Ice Age.

Diels (131) points out that a geographical criterion may be particularly valuable when a group is adapted to a definite set of environmental factors, since such factors are nearly always spatially differentiated. The dependent groups must then show a corresponding distribution in space. If a group of systematic units be in a high degree epharmonically or adaptively organized, its geographical distribution can be used to test whether or not the morphological criteria have phyletic significance. The geographical method is not limited to clarification of simple epharmonic form-circles. For more difficult groups the method is essentially the same as for the simpler: firstly, the series of morphological progressions are established and the distribution of the races mapped, then the geographical point of origin and the migration routes of the existing races are determined, the characters are compared with the environmental conditions of the ranges, or with their postulated past

conditions, and finally the morphological gradation is made to accord with the geographical distribution.

Smith (403) has published a paper on the Bromeliaceae which gives an excellent idea of the use of geographical data in taxonomic and phylogenetic studies. Smith frankly states that morphological facts must be given primary and range data secondary value.

Distribution of the higher systematic categories, especially genera and families, has been relatively little investigated on modern lines. Attention may be called to the paper of Guppy (175) and the work of Irmscher (240). Guppy concluded that the history of the angiosperms is to be resolved into two principal eras: that witnessing the rise of the great families, under relatively uniform environmental conditions, and that in which the family types were differentiated in response to the differentiation of climatic and other conditions. His statistical treatment shows that the distribution of families largely ignores the cleavage of the land into two great masses diverging from the north, but agrees in a marked degree with the differentiation of climatic zones. Guppy's paper was read in February 1918 and he does not refer to Wegener's theory. His views on the relation between mutation and natural selection need considerable modification in the light of recent researches. Willis (492) accepts and extends Guppy's theory of differentiation. Irmscher throughout his work most strongly supports the Wegener hypothesis. He concludes that the following three factor-complexes have acted together to give the present distribution of flowering plants: "1. Polverlagerungen als Ursache der Pflanzenwanderung und Florendurchmischung. 2. Grossschollenverschiebung und damit im Gefolge Veränderung des Grossformenbildes. 3. Aktive Ausbreitung und Weiterentwicklung des Pflanzenbestandes". Similar conclusions are reached as a result of a study of the distribution of mosses. In reading Irmscher's work one feels that his regional divisions are far too large—there are only four for the whole land surface of the globe. Tabulations on such a basis are very easy to construct but entirely obscure many of the most important correlations between range on the one hand and environmental and historical factors on the other. That phytogeography has much light to throw on phylogeny and much help to give to taxonomy, the present writer firmly believes, but full and careful analysis is essential before synthesis.

(*The concluding part of this study will appear in the December issue of the Review*)

ECOLOGICAL PROBLEMS OF THE SOUTHEASTERN UNITED STATES COASTAL PLAIN

B. W. WELLS

North Carolina State College

Geographically the southeastern United States coastal plain may arbitrarily be delimited by southern Virginia and the Mississippi River. In width it varies from 115 miles on the Virginia-North Carolina line to nearly 600 miles in the Mississippi basin. It involves the southern part of the geographers' (17a) Embayed Section of the plain, extending to Cape Lookout; the Sea Island Section, extending to the Florida (peninsula) Section; and the East Gulf Section extending to the bottom land of the Mississippi, which is separated into an Alluvial Plain Section.

Physiographically it may be divided into sea terraces of which the following six are commonly recognized, beginning at the piedmont border: the LaFayette, Coharie, Sunderland, Wicomico, Chowan (Talbot) and Pamlico. These terraces record the Pleistocene changes of sea level believed to have been due to volume changes in polar ice masses associated with glacial and interglacial periods. The upper coastal plain may, however, be largely fluvial in origin rather than marine (18). The older or upper three terraces have been eroded into an uneven topography with enough relief to drain the uplands, while the lower three are largely in a flat condition of low relief with emphasis on lack of drainage. Variation in rate of water movement of the earlier ocean and sounds has resulted in local variation in sand textures, a basic factor of the utmost importance in later determination of upland vegetation.

Climatically the area is characterized by a uniformity unusual for a region extending nearly 900 miles in a north and south direction. Most of the coastal plain has mean annual temperatures between 60° and 70° F. Only the lower half of Florida goes above this. Other significant temperature data (37a) follow: The average normal daily temperatures for the coldest 14 days of the year range from 40° southward to 60°. The absolute minimal temperatures range from slightly below 0° F. southward to 14° F. in middle Florida. The number of days of the average frostless season ranges from 220 southward to 320 in middle Florida. The

normal annual precipitation range is from 45 to 60 inches with most of the plain occurring in the 50–60 inches zone. The number of days with normal precipitation of more than .1 in. in a period of average frostless season ranges from 175 to 225. The precipitation-evaporation ratios, 1.0–1.4, based upon the average frostless season, characterize most of the area. The normal mean aqueous vapor pressure range for the average frostless season is from .55–.60 in. for practically the entire region.

Vegetational response to these edaphic and climatic conditions has resulted in a remarkable mosaic of plant communities, both successional and climax, a mosaic so real that air photos, particularly the index ones, covering a county, show far greater diversity than anything to be seen in the piedmont and mountain areas.

Under so uniform a climate, this ecological richness is largely due to topography and soil textures decreasing or increasing the soil water to extremes—the rolling sandy uplands are too dry (sandhills), the flat uplands are alternately excessively dry or excessively wet, according to season (shrub-bogs, pocosins or bays and grass sedge bogs or savannahs), or the lowlands are flooded all or part of the time (marshes and swamp forests). Profound and important changes in nutrient occur with the coarse textures, changes which may play a major role in determination of the plant community. Succession would simplify the mosaic just outlined considerably were it not for fire. Winter and spring fires are as much to be expected as summer rains, with the result that over vast areas certain successional communities are highly stabilized and constitute fire climaxes or serclimaxes of Clements (12a).

Fire in the coastal plain is more common than in the adjoining piedmont because the herbaceous vegetation of the sandy uplands and the foliage of the shrubs in the extensive bogs have a higher fiber content than the corresponding vegetative structures of the more mesic vegetation which grows successionally or as a climax in the piedmont area. In drouth seasons the upland communities may burn freely at any time of year, with the greatest incidence in winter and early spring because of the absence of new growth and the custom of the natives to burn the "rough" to improve pasturage.

The present-day student of coastal plain ecology must keep in mind that during historical time drainage has permanently lowered water tables over large areas. This has resulted in changes of

vegetation and soil surface conditions which have favored fire. The great increase in fire incidence in the last 50 years must be in part credited to this fact.

In a review such as this it would be impossible to discuss all communities and their variables. What follows is a presentation of the most important stabilized vegetations (associes and associations of Clements (12)) together with a discussion of environmental conditions which account for these stabilizations on the basis of present knowledge.

The only major communities which do not fit into the above general picture are those in the immediate vicinity of the sea where the factor of salt spray has been shown recently (62) to be of prime importance. These will be dealt with first.

SALT SPRAY CLIMAXES

On the southeastern coast of the United States, the discovery has been made that wind does not produce the one-sided malformed shrubs and trees (61, 62), comparable to the wind repressed woody plants at high mountain timber lines. Along salt water shores where surf action is vigorous enough to throw salt spray in the air, the higher winds carry the salt spray ashore where it directly and quickly blights the young shoots of woody plants exposed on the windward side. Those on the leeward are uninjured, since for the particular spray intensity zone endured by the plant, the sea-facing branches strain the spray out of the air so that the leeward shoots remain uninjured. Further, with low trees and shrub masses the current of spray-free air which rises behind the obstructive plant, tends to protect the leeward shoots. Frequent shoot pruning on the seaward side with uninhibited growth on the leeward fully accounts for the extreme malformation of coastal woody plants. Shrubs and trees exposed to sea winds but far enough from the strand to be out of the spray zone, show no such streamlined forms.

Confirmation of these observations comes from South Africa (5) where on the coast of Zululand deformation of the trees is "Due to the effect of salt-water spray which kills off the young shoots on the exposed side of the trees". The role of salt spray is of much greater significance than this, for it is evident on the North Carolina coast that a definite zonation of vegetation occurs in relation to spray intensity. The sea oats (*Uniola paniculata*), which domi-

nates the sea front dunes, is a highly successful spray-resistant plant with its coriaceous ridge and groove leaves.   Before the spikelets emerge from the enveloping leaf sheath, the two pairs of protective glumes and the lemmas develop outer layers of dead lignified cells which give a high degree of protection from the spray.   In anthesis, only one of the two stigmas is extruded from the floret.

H. J. Oosting and Billings (41a) have shown the spray-sensitive grass *Andropogon* to be confined to dune pockets where spray-catching cloth squares showed little salt carried in contrast to those placed on exposed points.   This quantitative study of salt spray intensity is the first made, and extended use of this method should prove important in attaining a much more accurate understanding of the role of salt spray in plant distribution.

In the medium spray zone, wax myrtle (*Myrica cerifera*), yaupon (*Ilex vomitoria*) and water bush (*Baccharis halimifolia*) are important shrubs which are associated with one dicot tree, the live oak (*Quercus virginiana*).   It is this resistance to salt spray injury which seems to account for the live oak's attainment of almost pure dominance on exposed islands (Smith Island, N. C.) (63).   Wherever freed from competitors by the medium spray which it can resist, this slow growing oak becomes a really important tree on the mainland in the vicinity of the sea (45) and Gulf.   Its dominance along the shores of the latter is indicated by the statement: "Of the magnificent groves which once lined the shores of the Gulf but few remain" (41, 42).   The palmetto is equally spray-resistant and is to be frequently seen in the coast live oak communities.   The slash pine (*Pinus caribaea*) has been observed by the author to be very resistant to salt spray.   This is also supported by data from Horn and Cat Islands, Mississippi (42, 48).

The details connected with the nature of the salt injury are yet to be worked out.   From superficial observations so far made, it seems necessary for the salt to enter the adult leaves in such high concentration as to rapidly withdraw water from the cells.   The young leaves and stems, with thin cutins, are probably blighted by the external salt solution.   During the higher winds necessary to carry the spray inland, evaporation of the water on the leaves would inevitably bring about a very high concentration of the saline material, in contrast to the original 3% concentration of sea water.

From these preliminary observations it is evident that along

shores throughout the world, salt spray is of major importance in modifying the form of woody plants and in developing a zonation of vegetation related to salt resistance, the communities of which persist for centuries and thus constitute climaxes, dune-grass, shrub and forest communities, differentiated into zones by salt spray intensities. These spray-stabilized communities are characterized by a highly restricted number of species, the taller and more exposed of which show evidence of protective leaf structures, as thick cutins, thickly massed simple trichoness (*Croton punctatus*) or stellate ones (*Quercus virginiana*).

These salt spray stabilized communities are especially interesting because of the problem they become in attempting to fit them into a monoclimax concept of succession. Stabilized by different salt intensities, the grass, shrub and live oak forest communities are all climax on a stable coast line. And as such they may be dealt with satisfactorily only by a polyclimax scheme of classification.

AQUATIC AND MARSH COMMUNITIES

A prevalent concept in aquatic ecology is that water depth is primarily in control of the communities, with these zoned in a simple sequence from the shore outward. This is shown to be misleading from observations made on lakes and ponds with sandy bottoms. Texture of the substratum appears to be more important than water depth (6). On pure sand *Eriocaulon compressum* is a common pioneer and may frequently be seen growing in but a few inches of water as well as the deeper water of 20 to 30 inches. Eventually the accumulation of organic matter (in many ponds a very slow process) will change the bottom so that rooted aquatics (*Nymphaea, Castalia*) followed by the marsh herbs may come in. One marsh grass (*Panicum hemitomon*) is notable for its ability to pioneer in the sand, but it always appears in a weak open stand.

Once the soil habitat has changed to an essentially organic or peat type, then the depth factor probably becomes of major importance.

In the middle Atlantic region many lakes are found in some of the puzzling "Carolina Bays". These latter are very shallow depressions with definite sand rims forming almost perfect ellipses and are found indiscriminately scattered over the flat interstream uplands of the lower plain. Since these elliptical depressions, now

mostly filled with peat, were originally lakes and ponds with an aquatic flora, the physiographic problem of their origin may properly be presented here.

Physiographers are still vigorously debating the origin of these depressions, most of which originally must have been dominated by aquatic communities. Were they made by solution (34a), water movement (13a), or are they the common result of the catastrophic impact of a great mass of meteorites (40a)? From extensive field acquaintance with many of them and a study of the recently available air photos of the region, it is difficult for the writer to believe that the remarkable regularity of their perfect elliptical outlines could ever have been produced by such variable factors as underground (solution) and surface water movements (13b).

In these bays, however formed, the hydroseres show great diversity due to wide variation in the behavior of the water table.

Most of these hundreds of bays have been filled (or nearly so) with peat. Curiously enough some have not, and these contain dark water lakes, with one remarkable exception in White Lake of Bladen County, North Carolina. Presumably the meteorite in this case broke through a stratum of rock releasing a large flow of water which, as in an artesian well, has been pouring upward into this nearly two mile long depression. The sparkling clear water of this lake in contrast to the dark waters of the neighboring ones, contains, according to L. A. Whitford (data not published), a very meager plankton. Both the number of species and the number of individuals represented are but a small fraction of those in the dark water bodies. The reasons for this contrast are still to be worked out. We have here what might be designated a sterile aquatic habitat.

One striking peculiarity of all the bays still showing open water is the low amount of marsh vegetation in them. With shallow water, particularly on the southeastern side, they show no extensive development of the familiar marsh communities such as cattails (*Typha*) and wild rice (*Zizania*). The explanation, in part, lies in the fact that occupying upland sites as most of them do, the water table is a fluctuating one with the result that in the drouth phase of the decade or greater cycles, any marsh development is readily invaded by the ligneous plants and held indefinitely.

In eastern North Carolina and along the Gulf coast are the great

marshlands of the United States, yet they are very imperfectly known. The ecological problem is complicated in these localities because of the transition from fresh to salt water. The most important report (43) which comes from the Gulf region gives *Typha* and *Scirpus* spp. as the chief fresh water dominants with *Cladium* in weak brackish water (.5%). *Juncus Roemerianus, Spartina cynosuroides, S. patens* and *Distichlis* and *Phragmites* are the dominants in brackish water (.5–2.0), while *Spartina alterniflora* and *Distichlis* are the herbaceous marsh components of the salt water (.2–5.0%) habitats. Fire may change a *Spartina patens* community to one dominated by *Scirpus robustus* or *S. Olneyi*. J. C. Rabb, working on a North Carolina Department of Conservation project, finds fire changing a *Cladium* consocies to one of *Scirpus Olneyi*. One often finds two dominants in immediate juxtaposition in the marshes, with no ecotone, a situation for which fire may be chiefly responsible. The time of burning is doubtless very important, for a present dominant may be more severely set back with a spring fire running through the old debris after the plants by starting growth have exhausted the food reserves of the rhizomes. The few observations made on fire in marshlands definitely indicate that marsh communities in their perennial wet habitat are still subject to major changes under impact of fire. Far more observations must be made before we may definitely ascertain what are fire serclimaxes as these are distinguished from normal successional consocies.

One of the greatest developments of fresh water marshes characterized by a single dominant is that of the vast saw-grass (*Cladium*) community of the lower Florida everglades (29). For extent and single, almost pure dominance, this is one of the most remarkable communities in the world.

An initial community of the floating mat type (sphagnum or sedge) of the northern pond hydrosere, is not recognized in the South.

SWAMP FOREST

This community is one of the most extensive and distinctive of the typical southern vegetations. The true dominants are limited to three trees, cypress (*Taxodium*) and the two gums, swamp gum (*Nyssa biflora*) and tupelo (*N. aquatica*). Since the seeds of two of these species have been shown to be incapable of germination

under water (16, 50), it is clear that the familiar surface water of these forests cannot always be present. It is now recognized that the southern swamp forests occupy areas characterized by a fluctuating water table. The extreme drouth which could lower the water table to the soil surface in the deeper swamps, may occur only at 10- to 20-year intervals. This requirement of gaseous oxygen for seed germination makes it possible to read the record of maximum depression of the water level in our ponds and stream basins, for this level, if cypress is present, will be marked by the outermost trees.

In contrast to the areas of water table fluctuations, are the marginal coastal fresh water bodies where the ocean level prevents fluctuation. It is interesting to note that such open shallow water sites are occupied by extensive marshes. The swamp forest cannot invade here, since the seeds lying under the water will never be exposed to gaseous oxygen.

Not only the seeds of cypress require oxygen but the young plants as well (16). Plants as high as 30 cm. could not stand submergence. This means that establishment occurs only during years of exceptionally long drouths.

The southern swamp forest tree dominants have flaring or buttressed bases, and, in addition, the cypress has its peculiar knees. By some (40) these structures are believed to be of mechanical significance rather than devices for increasing root respiration. Growing as most of these trees do in a soft yielding substratum of deep silt and muck, it is notable that they are seldom seen in a windfall. The cypress knees, however, are demonstrably a structure involved in respiratory gas exchange, so in the cypress these structures have a dual function, anchorage and gas exchange. The gums (*Nyssa biflora* and *N. aquatica*) develop small knees but these are not so numerous as those of cypress (22a).

The expanded bases and the knees seem to be a response to the combined stimuli of water and air at the water surface (36). Upland cypress does not develop these structures. In Reelfoot Lake, in which a marked artificial elevation of the water level occurred, the cypress trunks responded at the water surface by developing new swollen zones.

Cypress is reported in the role of pioneer in the Carolina Bays (7). Becoming established in the shallow marginal zone during

low water periods, the flaring bases of these trees later become island foci of spreading shrub growth which when massed may extend a few feet from the solid substratum out into the surface water. This pioneer role of cypress is confined to the shallower portions of the lakes, since these alone will be exposed in the depressed stages of the water table cycle. The initial peat accumulation of the deeper areas must have been carried out by the shrubs alone, since aquatic and marsh vegetation is very weakly represented in the impounded waters of the Carolina Bays.

It is of interest to record that in calcareous waters, as are found locally in Florida, the composition of the swamp forest is much the same as in acid swamps (24). The author concludes that "The amount of fluctuation of the water table is more important to some plants than the chemical properties of the soil". Tupelo gum is reported in water having a pH of 7.1 (44).

In this discussion "swamp forest" has been restricted to areas normally covered with water and the correlated trees.

The white cedar (*Chamaecyparis thyoides*), often treated as a swamp tree, is not so regarded here, since it is a tree of peat bogs where open surface water is not the normal condition. It is best dealt with in relation to the shrub-bog community with which it is involved in a successional relation.

PEAT BOGS

The southeastern coastal plain contains the largest peat areas in the United States. The Florida everglades, the Great Dismal and Okefinokee Swamps, Angola and Holly Shelter Bays of North Carolina together with the great Dare County peat area are a few peat masses each to be measured in hundreds of square miles.

Active southern peat areas are best classified primarily as upland or lowland peats with the latter including marsh and swamp forest peats which will not be considered in the following discussion.

The upland peat areas or bogs may be satisfactorily dealt with under two community heads, shrub-bogs and white cedar bogs, the former being of vast extent and the latter of highly limited extent and distribution. By the term "upland" as used here in relation to the lower terraces of slight relief, we mean non-flooded interstream areas in contrast to the flooded lowlands of marsh and swamp forest sites. The smaller stream margins, together with

those of swamps and lakes, may carry peat bogs and are here included as "upland".

On poorly drained flat interstream areas of the lower terraces, areas which include the Carolina Bays, is to be found one of the South's most distinctive communities, the "bay," "pocosin" or evergreen shrub-bog. From a research point of view it is one of the most neglected; no detailed habitat studies have yet been made of these peat areas. Under the prevailing condition of universal fire, trees are at a minimum except for the "pond" or "pocosin pine" (*P. serotina*). Hence these bogs have been of little interest to foresters. Further, the impenetrable vegetation and the uncertain peat substratum normally does not facilitate field studies of any kind. This interesting community is one which is not simple but which is represented by a number of dominants. The most important of these are *Ilex glabra, I. lucida, Zenobia cassiinifolia, Arundinaria tecta, Magnolia virginiana, Cyrilla racemiflora* and *Seronoa repens*. Of these, cane (*Arundinaria*) was formerly much more abundant than at present (27).

It is to be noted that most species found in this community are characterized by coriaceous leaves, having shapes which are minor variations of ellipses. Many are heaths and thus are similar by family relationship, but others, like the omnipresent "bamboo" (*Smilax laurifolia*), vary from the genus type of heart-shaped leaf to the elliptical.

Shrub-bogs are to be seen in most of the Carolina Bays. These have become filled with peat which brings their surfaces few to many feet above the bottoms of the nearby lowland swamps. A few very large ones occupy broad interstream areas with a very shallow depression which has been filled with peat, making the present site, like those of the bays, an upland one. This fact is important, for it means that during wet seasons the water table stands at or near the surface, while during dry ones the water table falls so low that the surface peat layer may dry and burn. These plants are thus alternately exposed to low soil oxygen and low soil water with its consequent high soil oxygen. Marsh and swamp plants are kept eliminated by the latter, and upland plants by the former. What the adaptations are which enable these shrubs to survive both of these extremes during a single summer are not

known. Particularly how these shrubs survive the long hydro-periods (high water table) with low soil oxygen is not known. And the morphological and physiological leaf adaptations to the drouth periods are equally unknown. Under southern conditions, marsh gas would be excessive, and according to one hypothesis (3) deserves further study.

A correlation to be dealt with more fully under the Longleaf Pine Subclimax may prove to be equally valid here. The uniform high acidity of shrub bog soils (pH 4.5) is, of course, indicative of low calcium, a nutrient condition to be correlated with a high carbonaceous state of the plants (2). Anatomical studies (McMenamin, unpublished) show these leathery leaves to possess an unusual amount of cellulose and lignin deposited particularly as sclerenchyma sheaths around the veins.

In this community as in others in the coastal plain, stabilizing fire is ever present. Root crowns commonly are able to renew shoot growth. But new growth seldom goes beyond five years without fire reducing it again. Solution of the problem as to what will happen without fire must remain largely hypothetical, for demonstration areas are practically unknown in the South.

WHITE CEDAR BOGS

White cedar (*Chamaecyparis thyoides*) has a very diffuse and irregular distribution (26) in the coastal plain. Only a few stands remain, scattered at wide intervals where they commonly occur on upland peats. The largest stands were in Virginia and North Carolina. M. F. Buell (unpublished data), from observations made on white cedar stands in southern North Carolina, holds that these stands constitute a response following fire which occurred at a time of sub-high water when the raw surface peat is not destroyed. It had been earlier pointed out (35) that, in the management of white cedar areas, burning the upper layer of peat results in establishment of either pond pine or swamp black gum and red maple. A completely exposed peat area with a persistent sub-high water table plus sufficient seed either from trees or stored in the peat, seems to be the important set of conditions bringing about initiation of a white cedar stand (1). The young trees may encounter much competition with the shrub-bog vegetation. "Bamboo" (*Smilax laurifolia*) will often be one of the worst competitors because of its

habit of spreading thickly over the entire young tree, checking its growth completely. Those initial stands in which the trees are very closely spaced, developing heavy shade in early stages and thereby checking the shrubs, will mature into the best old stands.

Once established, the community, if destroyed by fire, may regenerate from seed, provided fires are not too closely spaced. Without fire, Buell finds that old white cedar stands give way to a subclimax forest of red bay (*Persea*), ti-ti (*Cyrilla*) and sweet bay (*Magnolia virginiana*) trees, of which red bay is the dominant, and 90% of the forest floor plants is of this species.

White cedar forests may be regarded as special fire serclimaxes, the special conditions occurring so infrequently on the great peat areas of the South as to highly limit the extent to which these forests have appeared.

SAVANNAHS

Savannahs, grass-sedge communities with or without trees, are widely spread on the lower and flatter three terraces of the coastal plain. Those without trees are often called "prairies". Because of many typical bog plants (*Sarracenias*), together with so many soil characteristics similar to those in true bogs, these areas are most accurately described as upland grass-sedge bogs, even though they typically exhibit a mineral soil. The grass-sedge communities on them are mostly of recent origin in the historical period due to destruction of an earlier shrub-bog vegetation under higher fire incidence of the white-man era. Everywhere relict shrub-bog plants, particularly *Arundinaria,* may be found in them, indicating their place in a retrogressive succession. Grasses dominate these areas even though a number of species of pine are found on them in open stand.

The only savannah community seen on peat is that of the bunchheaded broom-sedge (*Andropogon glomeratus*) where it had come in after destruction by fire of an extensive stand of white cedar.

The two most important dominants are orange or tooth-ache grass (*Campulosus aromaticus*) and wire-grass (*Aristida stricta*), the same grass represented so widely in the sandhills. The former is the indicator of fine sand texture, while the latter is always associated with a medium to coarse sand texture. On better drained sites, *Panicum virgatum* and the common broom sedges (*Andro-*

pogon) may appear. Various sedge genera, for examples, *Ryncho-spora, Fimbristylis* and *Scleria,* are well represented but seldom if ever assume dominance. All these plants would be classified as xeric, having thick cutins and narrow leaves; on the forbs (herbs other than grasses and sedges) the leaves are appressed against the stem. But most significant is their high fiber content. So great is this that despite the attractive appearance these prairie-like grasslands make as possible forage areas, they are useless for grazing except in spring before the indigestible carbonaceous materials (cellulose and lignin) have been deposited in excess.

The complex which is to be correlated with this vegetation as controlling is as follows: Though broad and flat the habitat is an upland one which in extended drouth periods has a low soil water content. The subsoil is non-draining (commonly hard pan under wire-grass) which causes, during periods of frequent rain, a persistent high water table making a water-logged soil low in oxygen. This community is comparable to the typical shrub-bog in that the plants are adapted to surviving the extremes of soil water. These sandy soils long ago had their calcium content lowered by leaching, a factor which is to be correlated with the high carbonaceous condition of these plants (2). The highly combustible nature of this vegetation increases fire incidence so that these grass-sedge bog or savannah areas commonly burn every year. Many of them have become highly stabilized as fire serclimaxes.

Connected with fire, the interesting observation has been made on the North Carolina savannahs that swamp gum (*Nyssa biflora*) and sweet bay (*Magnolia virginiana*) may persist for many years by developing annual shoots from semi-buried short stumps which date back to a presavannah period. Such fire-repressed trees which must have an age of 80 years or more have been seen (58).

Other soil characteristics may be illustrated by those of a North Carolina savannah studied intensively (58). The following data were obtained from this soil, a Portsmouth very fine sandy loam: Surface soil porosity of 12.5% with the water table at 1 ft. is contrasted with a 33% porosity with a 5 ft. water table. Dark humus layer 14 in. thick. pH, surface: 4.4; 6 in.: 4.6; 16 in.: 5.0. Organic matter surface soil, 5.4%; total carbon 3.1%. Oxygen content of water from 5 in. depth (high water table), .3 ppm. in contrast to 7 ppm. of same water after shaking in presence of oxygen.

Total nitrogen about four times that of neighboring fields. Nitrates none. Ammonia present and constituting source of nitrogen for the plants. Bacterial content very low. Actinomycetes present but under low oxygen condition of hydroperiod, lignin decomposition function low. The significance and role of the low calcium content has already been mentioned.

It has been found that the rate of fall of the water table is highly important in determining the composition of a savannah community (58). Areas having a slight slope or a small amount of subsoil drainage, making possible a decrease in the time of surface water logging, show a very different composition in subdominant and minor species. So striking a response is this, that the plants may with great accuracy be used to interpret drainage conditions. These facts mean that in these areas the problem should be dealt with in terms of length of exposure to waterlogging. This factor, the "hydroperiod" (58), is of major importance in studying the varying composition of savannah communities everywhere.

The general depression of water tables brought about in recent time by drainage, and the more frequent fires of the white man, have greatly increased the areas dominated by herbaceous savannah communities. This increase has been largely at the expense of shrub-bog communities, communities on thin layers of peat which was destroyed along with the shrubs. This retrogressive change from shrub-bog to savannah is to be recognized as one of the major vegetational replacements of the historical period.

Before leaving the discussion of the savannah community, a word of caution should be given the casual ecological observer relative to temporary shifts in dominance and aspect due to seasonal variations in rainfall. On a North Carolina savannah under close observation (58) the area during a summer which followed a very dry spring, changed aspect completely by the appearance of *Andropogon furcatus* in a heavy stand. In another season characterized by excessive rains, a less striking change was produced by the appearance of *Rynchospora Chapmanii* as a frequent local dominant.

LONGLEAF PINE FIRE SUBCLIMAX

Because of the forest value involved, the longleaf pine sandhill community has been studied more extensively than all the other southern associes grouped together, to the end that a fairly clear picture of the plant-factor interrelations has been obtained.

Longleaf pine (*Pinus palustris*) may be accompanied by scrub oaks (*Quercus Catesbaei, Q. Marylandica*), low shrubs (*Gaylussacia dumosa, Ceratiola ericoides*) and characteristic herbs (*Aristida stricta, Tithymalopsis Ipecacuanhae*). The kinds and quantity of understory plants are related to fire frequency and intensity, the high-fire forests being very low in the minor woody plants with the perennial wire-grass and other herbs tending to persist.

One of the most significant characteristics of the ground cover vegetation and one not sufficiently emphasized in the literature is the high fiber content of these plants. It is the high proportion of cellulose and lignin in these plants which accounts for high fire frequency in these forests. And the dried, fallen pine needles with an equally high ligneous condition, merely enhance the tinder box nature of the sandhill ground cover.

Wire-grass (*Aristida stricta*) should be singled out for special attention. One study (60) presents the anatomy of the wire-grass leaf blade, showing the body of the blade to be made of heavily lignified fibers, the living tissue being practically limited to narrow bands of chlorenchyma, one cell thick in the ridges (ridge and groove type of blade) together with a small strand of phloem in each ridge. The mature living wire-grass leaf is thus but a slender sliver of wood which explains why tussucks of this grass may burn easily three hours after a rain.

A recent suggestion, already referred to, (2) may prove to be of extreme importance in sandhill ecology, since it offers an explanation for the high fiber nature of sandhill plants. It is pointed out that calcium is correlated with high protein, and potassium with high carbohydrate; thus a marked imbalance between the two, as obtains in the medium to coarse sand soils, may result in an extreme shift in the protein-carbohydrate content of the vegetative structure. Due to low nutrient adsorption of the coarse textured sandy soils coupled with excessive leaching action under high coastal plain rainfall (45–60 in.), the calcium content is extremely low, as indicated by the typical sandhill acidity of pH 4.5–5.0. The calcium disappearing more rapidly than the potassium results in an extreme shift to carbohydrate and lignin plants, a shift made in the phylogenetic and ontogenetic adaptations of these species.

This concept should, of course, be checked experimentally, using native vegetation, before it may be finally accepted. It, however,

seems to fit so perfectly into the picture of the sandhill-pine complex, that its validity would seem to be foreshadowed.

Before leaving the discussion of high fiber-fire relationship it should be pointed out that longleaf pine is an exception in this picture with regard to fire. With leaves high in fiber this tree is, nevertheless, remarkably resistant to fire. Longleaf branches have survived after an eight minute exposure to fire (4). The radiating needles, not easily ignited, constitute a veritable armor against injury to the young stem. The resistance offered by the bark of older trees is too well known to need emphasis.

There is another way (60) in which nutrient enters the sandhill ecological picture, and that pertains to the explanation of the well known xeric nature of sandhill plants.

The field capacity of these soils is relatively low, and in light of the fact that roots must grow to maintain water absorption, the extremely low nutrient content must tend to inhibit root growth and thereby inhibit water intake. Low transpiration during warm drouth periods is to be correlated with this low intake and reflects the high degree of xeric adaptations observed in these plants, adaptations to be regarded as distinct from the high fiber response discussed earlier.

The high degree of xerism in sandhill plants is further emphasized by the root distribution of wire-grass which has over 95% of its roots in the upper 6 in. when growing in deep medium sand (60), and with longleaf pine the lateral roots (about 90% of the root system) occupy the first foot of the soil (30). In longer drouths the water content of this superficial layer drops below the wilting point, yet these plants survive.

It is evident that both high fiber content and xeric adaptations of sandhill plants are to be correlated with low nutrient and low water-holding capacity factor intensities which in turn are correlated with coarse texture. A typical longleaf pine upland deep sand (60) gave a mechanical analysis of the first foot of soil as follows:

Fine gravel	2.70%
Coarse sand	17.66
Medium sand	52.48
Fine sand	17.52
Very fine sand silt and clay	8.86
	99.20

Typically associated with such a texture is the low organic matter content of .75% with a total carbon of .43%. The microflora is considerably below that of ordinary loam soil with the actinomyces number large in proportion to that of bacteria. The pH, 4.5–5. The carbon/nitrogen ratio is extremely high. No nitrates occur, and like savannah plants these obtain nitrogen from ammonia.

It is the medium sand which gives the texture character to most sandhill longleaf pine soils and is the most significant basic, indirect factor in control of the edaphic direct factors, which together with fire (to be discussed later) definitely determine the fire subclimax nature of the community. Where the texture shifts to finer sands or to colloidal silts and clays, the vegetational response shifts to more mesic lower fiber species with lowered fire incidence, resulting successionally in allowing progression to a hardwood climax.

On the finer sands (sandy loams) with the highest percentage falling between medium and fine sand, longleaf pine with the aid of fire, dominates vast areas (34), areas which without fire are invaded by such mesic species as the larger oaks, dogwood and sweetgum. On many such mesic areas where turpentining and lumbering have destroyed the longleaf, loblolly has assumed importance. Such a change has been noted in southeastern North Carolina where large acreages of longleaf have in historical time been changed to loblolly pine.

A special and remarkable habitat of the longleaf pine is to be noted on the more restricted flat areas where the special soil features are medium to coarse sand, underlain by a hardpan. Here it may be accompanied by wire-grass (60) or saw-palmetto (25). This type of soil condition means that the longleaf pine and its accompanying minor species are able to tolerate the low oxygen and other conditions created by high water in rainy periods. Experimental demonstration has been made of the survival of wire-grass for 10 weeks with a water table coincident with the soil surface (60). These facts that the longleaf pine occurs in such areas as well as on the well drained sandhills, show it to have a remarkable range of survival under extremes of soil water content. This seems to be equally true of the wire-grass. And where wire-grass is dominant it constitutes a savannah community, as earlier indicated.

With regard to the pines in general, lack of knowledge concern-

ing the role of their mycorrhizae in their distribution is probably a serious deficiency. They are known to be present on longleaf, slash and loblolly (46). The mycorrhizae of pond pine (*P. serotina*) have been believed important in making possible the growth of that tree in its characteristic peat habitat (4a). Probably no undeveloped field in southern forest ecology needs attention more than does this one. Greater knowledge of the ectotrophic mycorrhizae of the southern pines will probably modify many current concepts.

THE PROBLEM OF THE SANDHILL CLIMAX

Early vegetational maps and writings based upon the common observation of predominance of pines in the coastal plain gave the impression that pines were climax in this region. The "southeastern evergreen forest" was made coordinate with the "oak-hickory forest" of the piedmont region next to it.

It is now generally accepted that the extensive upland pine forests of the coastal plain constitute fire subclimaxes. This has not been easy to demonstrate because of the extreme paucity of areas unburned for 10 years or more. A few such have been found (34). On the well drained coarser sands xeric scrub oaks tended to replace the pines. On drained medium or medium-fine sands, southern red oak (*Quercus rubra*), dogwood (*Cornus florida*) and black gum (*Nyssa sylvatica*) were observed as definite invaders, indicating a final hardwood climax. On poorly drained flatwood sites, the pines were giving way to the competition of laurel and water oak (*Quercus laurifolia, Q. nigra*), sweet or red gum (*Liquidambar styraciflua*) and red bay (*Persea borbonia.*).

In North Carolina many early records show that extensive hardwood forests formerly covered areas in the lower coastal plain where today only pine is the common tree.

In the coastal plain even-aged pine stands will follow cutting of hardwoods, a succession not observed in the piedmont where pine groves uniformly indicate abandoned fields. This observation explains the rare cases of even-aged stands of pine covering large areas locally (56) which have been initiated by tornadoes in the coastal plain. These have been locally recognized as "hurricane forests."

The climax hardwood composition on various topographically and texturally different sites would undoubtedly be different—a series of consociations would result rather than one final monoclimax. The soil would to the last maintain a minor control.

THE PROBLEM OF LONGLEAF PINE FOREST MANAGEMENT

Foresters now recognize the chief natural checks on production of longleaf pine to be root-eating hogs (now largely controlled), the brown spot needle-disease fungus (*Septoria acicola*) and competition of understory plants.

The needle disease affects juvenile plants under two feet and may prove very important in preventing establishment of a satisfactory stand (51). It may definitely delay height growth (57). Burning of the affected needles by low intensity fire without destruction of the young tree (10) has been observed to check this disease.

Since 1849 (38) the great importance of fire in development of longleaf pine forests has been recognized. This, of course, is accomplished through freeing the trees from vigorous competition of scrub oaks, many shrubs, the high fiber wire-like wire-grass (*Aristida*) and a restricted number of other herbs among which the genus *Baptisia* is about the only legume to be mentioned.

During recent years studies have been completed which make possible an evaluation of vegetational and soil changes resulting from fire, fire which is certain, for no system yet developed has been able to prevent this type of destruction (17).

On the negative side it has been found that height growth is severely checked by frequent fires (8) and is even restricted by as much as one-fourth under controlled burning. Fire may destroy seedling pines and those at the 18-inch stage by injuring the young trunk. These are the two critical periods in relation to fire injury (34). Between those stages the radiating mass of fire-resistant leaves protects the trunk and growing point in a most efficient manner. In a close compact stand of young trees the rare crown fire may kill a large percentage of the trees. It must be recognized that fire during the growing season is more destructive than during winter (12).

The soils under burning become less porous because of destruction of the fauna (33), a change of structure which is probably of little significance in a coarse textured soil. No significant chemical changes produced by fire have been found which adversely modify soil fertility.

On the positive side of the picture as the vegetation is affected, fire removes the old debris cover, making it possible for seeds to contact the mineral soil and thereby definitely raise the germination

percentage (11, 21). Due perhaps to the higher calcium content, legumes have been observed to double their number (22). Early and vigorous growth in spring with debris removed makes possible a superior forage, cattle gaining 32 lbs. per head over those grazed on unburned areas (22, 55). Fire in relation to checking the serious brown spot disease has already been noted. Perhaps of greatest importance is the insurance value of the moderate frequent fire which by preventing accumulation of large masses of dead debris and thick stands of understory plants in the forest, prevents the holocaust type of conflagration which may be so destructive to living trees. But most important of all is the gain in longleaf pine production by eliminating the competitors of this tree (47), a tree which, according to all observers, cannot make its maximum growth under competition in the root zone where both water and nutrient are highly limited by coarse texture.

Soil improvement under fire follows from increased calcium content from the ashes which measurably lessens acidity. Higher phosphorus has also been noted (31). This change in nutrient, as indicated by a .5 pH acidity reduction, is probably not great enough to bring about any significant introduction of higher protein and less carbohydrate plants into the area. The soil humus, under fire, is added chiefly from grass root sources, resulting in increase in humus content (32). Fire does not affect the soil directly, since the temperature is not raised much above normal below one-quarter inch depth (32).

Though much is still to be learned about the fire problem, enough evidence is at hand to definitely prove that controlled burning in the sandhill regions is a constructive practice from the economic point of view (9, 52). Opinion will differ with different groups as to frequence and intensity. Cattlemen already burn grazing areas every year. Foresters (57) would probably prefer a three- to five-year interval, with a three-year interim for most sites perhaps the more desirable.

During the spring of 1941, an unusual drouth resulted in an extensive series of large sporadic uncontrolled fires in North Carolina which forced the State authorities to call into conference forestry men and others concerned, to see what could be done to prevent such losses in the future. From the survey summarized above, the answer would be that to put an end to the haphazard, destruc-

tive, uncontrolled fire, it will be necessary to initiate a program of constructive, controlled burning of established sandhill forests. In areas where juvenile trees must be protected through the crucial period, such control may be reduced to strip burning. Using an expanded and well organized fire warden personnel under the control of competent State authorities cooperating with the National Forestry Service, a carefully planned and well executed program of controlled burning should be carried out throughout the coastal plain area. Nothing short of this will adequately conserve the maximum economic returns from the vast acreages of non-agricultural sandy uplands in the South.

COASTAL PEAT DEPOSITS

These organic deposits are of especial interest, for recent ones confirm the physiographic theory which recognizes a recent rise in ocean level. Mangrove deposits in Florida (10 stations) show an average recent rise of ocean level of 7.4 feet (15). These deposits show a continuity which precludes the possibility of any marked reversal of level movement during the period of deposition. The mainland of Dare County, North Carolina, is covered by an extensive peat deposit, the surface of which is but a few inches above the surrounding sound level. Sampling along the eastern side of the area gave varying depths up to 12 ft. This deposit is continuous and may be compared with the Florida mangrove deposits. Both have increased in thickness as the water table slowly rose. Marsh peats of Currituck Sound showed a range up to nine feet with one restricted area showing a discontinuous deposit 35 feet deep below sea level (J. C. Rabb). On Smith Island, N. C., red cedar bases have been found hidden amid salt grass and shallowly buried in salt marsh silt in an area where this could have been due only to a slight but recent rise of the ocean level.

Older peat deposits which are unrelated to any living vegetation are abundant in the lower coastal plain. Many have been exposed by the landward advance of the ocean. Few studies have been made of these deposits and their interpretation in relation to former water table levels remains to be made.

One such in Carteret County, N. C. (14), with the base of the peat at sea level, shows in its profile, marsh, white cedar and aquatic shrub-bog in sequence. This peat sequence was compli-

cated by the area becoming ponded by development of dune sands on three sides exposed to sound waters. There are no data from this area which do not fit into the theory of ocean levels just described.

A profile of the Dismal Swamp peat (37) shows a similar fluctuation which the authors ascribe to a rise in sea level. Such a rise, however, would not be rapid enough to initiate retrogression. Impounding of water through final closure of a sand rim seems the more plausible for these local cases of retrogression.

One deposit was discovered by the writer which proved of special interest because it contained undecayed green leaves. This thin peat mass is located one quarter mile south of Wilmington Beach on the Cape Fear Peninsula. Extending for about 200 yds. along the beach it is exposed only after removal of the middle and lower strand sand by a strong storm shore current. The peat layer is 20 in. thick, the lower 4 in. being composed of a platy mass of leaves, leaves which were apparently blown from the trees in a hurricane and so quickly buried under water in the mass of organic debris that under the oxygen and light-free conditions they remained green for a few thousand years at least. This green pigment changed rapidly in bright light, dissolved readily in alcohol, and in the spectrum gave the characteristic chlorophyll absorption bands. The top of the deposit was about one foot below mean tide and carried numerous stumps of red bay (*Persea*) trees *in situ,* trees which indicate a water table not above the soil level. In this location this means that the ocean level has risen at least one foot since this leaf material was deposited, a change which must have involved a few thousand years, for physiographers are in agreement that the ocean level in recent time has changed very slowly.

In relation to any discussion of recent Pleistocene peats attention should be directed to the data (39, 49, 54) that indicate the last ingress of the sea to have reached a level 25 feet above the present. This level holds throughout the whole extent of the coast, indicating the absence of any crustal warping since it was established. Upon regression the sea retreated to a level certainly 10 feet below its present stand and probably more than this but not more perhaps than 25 ft. (13).

Correlation of the peat deposits with these recent changing ocean levels has been little more than initiated, despite the fact that such

evidence is available at innumerable points along the southern coast line.

REGIONAL CLIMATIC CLIMAXES

If we raise the theoretical question as to what the upland vegetation of the coastal plain would be after a thousand years without fire, only a hypothetical answer may be offered.

On the sandhill areas, as already indicated, the xeric and semixeric oaks (*Q. Catesbaei, Q. rubra*) and hickories would appear first related to a very slow but gradual increase in humus content with a consequent improvement in nutrient and water content. Whether such mesic species as white oak (*Q. alba*) could ever become dominant is an open question. Small white oaks transplanted into a typical sandhill have persisted and grown slowly. Certainly the highly mesic beech or maple would not be expected to appear on these areas in light of the fact that on the hilltop clay soils of the nearby piedmont region, the climax is definitely a mesic oak-hickory one.

On slopes made up of medium to finer sand soils rather than medium to coarser ones, beech, maple and tulip poplar have been noted in rare instances where roads, streams and fields in combination have furnished fire protection. Certainly on the drained medium to finer sand soils a full development of the eastern oak-hickory forest could be expected.

FLORIDA HAMMOCKS

Hammocks are isolated communities of hardwoods scattered in the monotonous stretches of everglade marshes and pine flatwoods. Shrubs and especially vines are abundant together with innumerable epiphytes. Typically the soil is "thicker than that of pine lands due to the accumulation of debris" (28). Some may be as small as an acre or less, though many are to be measured in hundreds of acres.

A common classification recognizes high and low hammocks, the former being on drier sites (29). These are further divided into types in accordance with the dominants. Live oak and palmetto types are prominent.

They have been definitely recognized as "fire islands" (53, 23), which means that they are protected from fire either by location or by including fire-resisting vegetation, and have survived as local

communities exhibiting great contrast to the dominating one of the general terrain. Under the greater fire frequency of the historical period, it is well known that large numbers of the smaller hammocks have been wiped out (59). But the general picture remains the same, *viz.,* local masses of mesic broad-leaved plants tending to maintain their community integrity against fires which periodically sweep through the more combustible vegetation surrounding them.

In arriving at a fundamental explanation of the more unprotected hammocks it is suggested here that Albrecht's concept of the correlation of high calcium with non-combustible, low fiber, mesic broad-leaved vegetation (2), is applicable. Large numbers of the hammocks have been noted to occur on "limestone reefs" (53) and one of the classifications emphasizes the calcium nature of the substratum by recognizing both "Calcareous high and low hammocks" (25). Further, where they are found on outcrops of finer sands and clays (20) the calcium-retaining properties of such soils would be the basis for maintaining this concept. Related to fire, the low-fire vegetation concerned would be the herbaceous and shrub cover.

Along the upper margin of the coastal plain where the sands change sharply to piedmont clays, the sharpest change may be noted from the high fiber, high-fire pine communities to the low fiber, low-fire hardwoods, a change which is always accompanied by a sharp shift in acidity indicative of the calcium differences in these soils. The hammocks, it is believed, are to be included in this general scheme of correlations, a scheme which has wide significance throughout the entire coastal plain.

The dominance of live oak and palmetto on many of these lower Florida peninsula hammocks may possibly be explained on the basis of selective action of hurricane distributed salt water spray. It is known that spray of killing intensity may be carried many miles inland by extreme hurricane winds, a spray which is capable of directly blighting most mesic leaves. As pointed out earlier in this paper, live oak and palmetto are remarkably resistant to salt spray. Though infrequent, the hurricanes with their salt spray-carrying power, should be recognized as a possible reason for bringing the live oak and palmetto to dominance on the Florida hammocks where they occur.

SUMMARY

The Southeastern Coastal Plain, composed of a sand mantle left

by regression of the sea, is characterized by at least six sea terraces. The lower three are dominantly flat in contrast to the more eroded and rolling upper three. The consequent extreme variation in drainage conditions plus the coarse textured (sand) soils constitute the most important indirect factors modifying the direct factor intensities.

Located in a region of relatively high and well distributed rainfall, one of the most important factor intensities, a resultant of this rainfall, plus the coarse soil texture, is the low calcium content. Applying the concept of Albrecht (2), this calcium deficiency is to be correlated with the high fiber and low water content of the herbaceous vegetation of the uplands. This in turn makes for greater fire frequency and intensity which greatly decreases the rate of humus accumulation at the soil surface, and by destroying the shade-producing shrubs and trees exposes the soil to direct radiation of the sun, thereby enhancing its already low water state. This in turn tends to maintain the low water state of the vegetation. Thus a vicious circle is established which explains the extreme degree of stabilization of the upland vegetation in the early stages of seral progress. In the historical period widespread drainage has greatly expanded these fire stabilized upland communities.

On the ocean front is a recently discovered kind of stabilization, *viz.*, salt spray communities which are zoned in relation to spray intensity, grass, shrub and forest, with live oak and palmetto the principal trees of the latter. Surviving where other trees are directly killed by the spray accounts for the local dominance of live oak near the sea and gulf shores. Further, the asymmetric or "wind blown" form of seaside woody plants is due to the blighting action of the salt spray on the windward side and not to any direct effect of the wind.

Marsh and swamp forest communities are to be correlated with stable and fluctuating water tables, respectively. This is due to the fact that cypress and gum tree seeds will germinate only in the presence of gaseous oxygen and not under water. The presence of swamp forest always indicates an area with fluctuating water table, the cycles of which, however, may be many years in length.

The upland peat areas are of great extent and are covered with either a dominantly evergreen heath shrub-bog or white cedar, the latter being highly restricted in comparison to the former. It ap-

parently is dependent on fire opening up the shrub-bog at a time of sub-high water table, for its initiation. A most important characteristic of the typical shrub-bog is due to its upland location and poor drainage. During wet periods the water table is high, at or near the surface, and during drouth seasons is low—so low that the peat dries above and burns readily. Shrub-bogs are abundant in the peculiar Carolina Bays, shallow elliptical basins, formed, according to the best substantiated theory, by the impact of huge meteorites in a single great shower. The leaves of most shrubs are coriaceous and relatively high in fiber. This is to be correlated with low calcium condition of the peat substratum (2).

The fire-made grass-sedge savannahs occur typically upon mineral soils (rarely on peat) and occupy a fundamentally similar habitat to that of the shrub-bog, *i.e.*, because of upland site and nondraining subsoil they go to extremes in soil water content in relation to the season. They show rate of fall of the water table (soil hydroperiod) to be a very important datum. A low calcium, high fiber, fire correlation is very prominent on the savannahs.

Recent coastal peat deposits indicate a slowly rising ocean at present. A unique older deposit is reported exposed on the sea front in North Carolina in which leaves buried under water in a platy mass by a hurricane, had been so sealed from light and oxygen as to result in preservation of the chlorophyll for a few thousand years.

So universal is fire in the area that examples of mature climatic climax communities are unknown. All evidence indicates that the extensive pine forests are fire sub-climaxes. The hypothetical suggestion offered for the upland climaxes is a xeric oak one for the deep coarse sand, mesic oak-hickory on the finer sand textures with beech-maple on the mesic slope bases. On still more moist sites but not wet enough to carry swamp forest, a characteristic community of red bay, sweet gum, red maple and sweet bay may be expected.

The Florida hammocks composed of hardwoods are interpreted here as communities having a fire-resisting low fiber ground cover correlated with a high calcium soil.

In this survey of the stabilized vegetations of the Southeastern Coastal Plain, nine well defined major communities (associes and associations of Clements) are described. In the differentiation of these the principal controlling conditions, aside from the over-all

climatic factors, are soil texture, as this controls water and nutrient (especially calcium), length of exposure to high water table (hydroperiod), salt spray (near coast) and fire, involving all except the aquatic.

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ECOLOGICAL RELATIONS OF PLANTS WITH ANTS AND TERMITES

J. C. TH. UPHOF
Washington, D. C.

INTRODUCTION

A close ecological relationship exists between certain plants and those groups of insects that have attained a high degree of social organization, namely, ants and termites. This relationship is often intricate and varied. In some instances these insects actually raise certain species of fungi for their own benefit. In other cases seeds are gathered by ants for certain purposes, which causes at the same time dissemination of the seeds and consequent distribution of the plant species. Certain plants harbor ants in their stems or in domatia on the leaves, thus giving shelter and in some cases furnishing food from certain bodies produced by the plants. A number of these instances may be regarded as representing symbiosis, some doubtfully, and others may be considered as parasitism.

The number of species of ant-plants is large. The most important observations were first brought from studies in the tropics where ants play a far more important rôle than in temperate zones. These plant-ants are widely known among the natives, and travellers and early botanists recorded them centuries ago.

Among these early known ant-plants are *Acacia cornigera* and related species. From an historical standpoint it is interesting to note that Francisco Hernandez (72), who was sent in 1570 by Philip II to study the resources of New Spain, found this plant in the Huasteca region of northeastern Mexico where it was called "Hoitzmamaxalli"; he named it *Arbor cornigera,* saying: "Generantur praeterea intra corniculos Formicae quaedam teneus, fuluaeque, & nigricantes". Later we notice how Commelin (33) in 1697 also described the ants in the thorn-like stipules of the same species which he called *Acaciae similis spinis corniformibus mexiocana.* His illustration is very clear and shows plainly those little

organs at the apex of the lower leaflets that are now known as Belt's bodies. Jacquin (81), too, in 1763 mentioned the ants on his *Mimosa cornigera*. Ray (130) in 1688 described the ants in the hollow stem of *Cecropia peltata* which he called *Ambaiba brasiliensibus,* stating: "In hac cavitate reperiuntum semper formicae rubrae". Ruiz and Pavon (140) in 1794 mentioned ants on *Cordia alliodora* in Chile and Peru, and Aublet (5) in 1775 described the myrmecophilous *Tococa guianensis* (Melastomac.) from French Guiana, which he called "nid de fourmis", there being ant-nests in the sacs of the leaves.

In the tropics of the Old World observations in those earlier days were not wanting. Rumphius (141), in his "Herbarium Amboinense", Liber III, 1741, observed in *Endospermum moluccanum,* which he called "Konings-Boom", that the stem and heavy branches have no heart and instead are filled with large black ants which work their way through the stem. At that time the Malayan name of the plant was "Caju summot", meaning ant-tree. Rumphius did not overlook *Myrmecodia* and *Hydnophytum* of which he gives one of the first descriptions and illustrations, calling them *Nidus germinans* or growing nests. He supposed that these plants originated from the material of the ant-nests, and considered them as "zoophytes" among the plants. *Myrmecodia* he calls *Nidus formicarum ruber* or red ant nest, and *Hydnophytum* bears the name of *Nidus formicarum niger* or black ant nest.

It can be easily understood that ants and termites which have "mentally" such a highly organized mode of life, which have their workers and soldiers, their egg-laying queens, their special method of feeding larvae, and their raising of aphids and scale insects as "domestic animals", necessarily must have a complicated and for us interesting method of gathering and using those parts of typical ant-plants that are eaten as food or used otherwise.

Studying the literature and comparing these ants and termites in their natural plant environment, one is convinced that much of the subject is still by no means exhausted for further investigations. A number of observations are too few or incomplete. Some conclusions have been made too hastily.

MYRMECOPHYTES

The term "myrmecophyte" or "ant-plant" is applied to plants inhabited by ants. The term is somewhat general and, considering

the different ways in which ants live with these plants, has given Warburg (183) reason to distinguish three different groups:

1. *Myrmecotropic* plants are those that provide food to ants in the form of sugary exudations (nectaries), certain food-bodies (bromatia), or seeds and fruits of the myrmecochores.

2. *Myrmecodomic* plants are those that give shelter to ant-nests in the form of hollow cavities (hollow stems) or in myrmecodomatia, swellings or pouches on leaves.

3. *Myrmecoxenic* plants are those that are true hosts, offering their guests shelter as well as food.

The word "myrmecophyte" was given by Warburg when he objected to the phrase "myrmekophile Pflanzen", stating "Wir wollen deshalb diese Pflanzenkategorie lieber einfach als Myrmekophyten bezeichnen"; he replaces the word "Myrmekophytie" for "Myrmekosymbiose", and "myrmekophytisch" for "myrmekosymbiotisch" which designates those plants that have distinct anatomical and morphological inheritable characteristics adapted to the use of ants. That plants receive in return protection from ants is in some instances not apparent. Several plant-ants do not offer sufficient protection against attacks of other insects because they are not carniverous. This is especially the case with those species of ants that prefer nectar.

It may be added that in relation to practical fruit-growing some carniverous species of ants have made themselves useful after they have been introduced into fruit trees by the aid of man. For centuries species of this group have been used in China for protection of orange and mandarine orchards. Also in some parts of Java nests of ferocious species are hung in mango trees. Dead leguans placed in the trees are furnished as food. To aid the ants in moving from branch to branch, bamboo-stalks are placed along various parts of the tree. In this way trees are kept clean by the ants from certain beetles harmful to the trees. Also in other parts of the tropics and in some sections of Italy similar practices are followed. One should also be reminded of the fact that *Formica rufa,* for example, though terrestrial, destroys many insects. It has been calculated that inhabitants of one large nest are able to devour 100,000 insects daily.

The ants that inhabit the large thorny stipules of different species of *Acacia* are decidedly of a fierce and savage nature and could be

considered dangerous to those coming close to the plants. This has frequently been recorded by various authors and I was also frequently witness of it in different parts of Central America. Similar conditions have been noted with *Bartera fistulosa.*

One has to consider the fact that several plant species possess hollow stems and are not inhabited by ants. It is supposed generally that the hollow stem serves a general mechanical purpose, giving strength to these plant organs. That ants have made use of them is another matter.

There is great difficulty, however, in explaining the direct use to the plant of domatia, leaf-pouches, *etc.,* in many species inhabited by ants. They are apparently not malformations caused by gall-insects or gall-fungi. They may be organs whose physiological function, if any, is thus far not clear. It is, however, difficult to imagine that they were specially "designated" for the use of ants. The same holds true for Belt's bodies on certain species of *Acacia* and Müller's bodies on *Cecropia.*

Myrmecophytes are found especially in those continents that support tropical vegetation. Most species are distributed in certain regions and are therefore described here geographically.

Myrmecophytes of America. The most widely known ant-plants in the New World are a number of *Acacia* species. Their connection with ant life was noted centuries ago. Schenck (154, 146, 147) and Safford (142) have given a description of these species, commonly known as "bull-horn" acacias. The most common is *A. cornigera.* Others are *A. sphaerocephala* and *A. spadicigera,* natives of Mexico, *A. cubensis* from Cuba, *A. bursaria* from Guatemala, *A. nicoyensis* in Costa Rica. Belt (14) was among the first to draw attention to these plants; he gave especially *A. cornigera* as an example. These plants were grown some centuries ago in greenhouses, especially on account of their horn-like stipules. Among the early collections may be mentioned that of George Clifford near Haarlem. A plant of this collection was described by Linnaeus in his "Hortus Cliffortianus" in 1737.

Among the ant species in these thorns are *Pseudomyrma Belti* var. *fulvescens* in *Acacia sphaerocephala* from San Luis Potosi in Mexico and *P. Belti, P. Belti* var. *fulvescens, P. spinicola, P. nigrocinta* and *Crematogaster brevispinosa* in *A. costaricensis.* The latter ant species may have occupied the thorns after they were left by *Pseudomyrma.*

These acacias offer the ants three attractions: (a) opportunity to build their nests in the stipules, (b) nectar produced by a crater-shaped gland at the base of the petiole, (c) small yellow pear-shaped bodies about 2 mm. in length at the end of the four or six lower pairs of the leaflets. Francis Darwin (35) described these bodies in some detail, and their use to the ants had already been described by Belt. These Belt's bodies possess a vascular bundle, continuous with the mid-rib of the leaflet, running at some distance into the food-body. The cells were found to contain strongly refracting oil-globules. Joki (82) states that the morphological construction of these Belt's bodies shows resemblance to that of glands, especially to epithem hydathodes of the leaf-apex. He believes that "die Beltschen Körperchen in ihrem heutigen Gestalt als Anpassung an die Ameisen aufzufassen sind".

Widely known as myrmecophytes are a number of species of *Cecropia,* especially *C. peltata.* These plants with their relatively large palmately divided leaves contain at the base of the petiole a flat cushion, 1 mm. high, embracing almost one-half of the petiole. It is protected by the sheath-like ochreae. This cushion is not found on young plants nor on the first leaves of small twigs. During development of the leaf the cushion has the appearance of a white, glossy, silky patch of unicellular hairs among which occur numerous multicellular hairs, soon present in the majority. They are composed of about 12 cells and reach about 1 mm. in length; they are milky-white when ripe and can be easily broken off. When dry they become yellowish. When not collected by ants, they spontaneously fall off. New ones are continuously being developed for weeks. These food-bodies, also called Müller's bodies, are much appreciated by ants. They do not contain any vascular bundles. In their cells is a granular protoplasm, as in those of *Acacia sphaerocephala.* Darwin describes in much detail the development of these food-bodies and considers them as of glandular origin. Schimper (148) interpreted the so-called Müllerian corpuscles, of much the same structure as those on leaflets of myrmecophilous acacias, as metamorphosed glands. Rettig (136) has called attention to the fact that similar bodies are found in plants not visited by ants. They may be a survival from a former symbiosis. Ule (178) is of the opinion that the carbohydrates and nitrogen substances contained in these corpuscles are not compensated for by the protection which the ants afford to the plants.

Blockwitz (20), who also studied these food-bodies, pearl-bodies and similar glands in detail, comes to the conclusion that the bodies of *Cecropia* originated from pearl-bodies (Perlblasen). Pearl-bodies are not glands or food-bodies; they are trichoms and serve for storage of water. Excretion of water is regulated by their sugar-content being gradually converted into fat.

According to Bailey (8), *Cecropia angulata* of the Kuitabo region of British Guiana is not colonized by its guest-ants until the plants have attained a large size. They are no more subject to defoliation by leaf-cutting ants, especially *Atta cephalodes,* than other plants. It is true that they prevent other ant species from visiting the upper part of older cecropias, but they do not appear on young plants. Ants obtain fat and protein from the Müllerian bodies and sugar carbohydrates for their coccids. Bailey states that "The ripe food-bodies are so assiduously collected by the ants that it is almost impossible to find one *in situ,* except on young uninhabited plants".

Fiebrig (49) made some extensive studies of *Cecropia peltata* in relation to the life of *Azteca Alfari* and other ants in Paraguay. This plant species is always inhabited by ants, except very young specimens. Ants of a particular tree must be considered as belonging to one large colony. The foundation of a new colony takes place in young trees, usually at the 10th to 20th internode, about one to two meters from the surface of the ground. Fiebrig found that this ant species has attained psychically a high degree of development. The Müller's bodies form without doubt the principal nourishment, especially for the larvae, and are produced by the plants in very large quantities. The ants apparently feed also on the fresh juice of the pith. Numerous insects were found on *Cecropia.*

In observing *Tachigalia paniculata,* Bailey found that in the hollow foliar axis are at least seven obligatory guest insects among which are ants and beetles. The inflated petioles of young plants are often occupied by young *Azteca* queens. The ants excavate pits in the walls of the rhachis and petiole, which become so deep that they perforate the epidermis. This plant does not offer food-bodies or protostomata, nor are there numerous extra-floral nectar glands. There is in the inflated petioles and the strands of parenchyma a kind of nutrition that has attracted the ants.

It is of interest to note that two sections of the genus *Cordia,* *Physocladia* and *Gerascanthus,* are furnished with myrmecodomatia. *C. nodosa* was studied by Bailey in detail. Its domatia are hypertrophied parts of the cauline axis, showing centrifugal and centripetal vascular tissues. Bailey describes their origin in great detail. Several species of ants were found in these pouches, among which are *Allomerus 8-articulatus, Azteca Ulei* var. *cordiae, A. inspabilis, A. trigonia, Crematogaster limata.*

Melin, in his studies on the theory of selection, mentions some myrmecophilous Melastomaceae from South America. He compares various views of different investigators for and against the myrmecophilous habit. The Melastomaceae in question are generally of shrub-like nature, growing near the outskirts of inundated areas; they also occur in firm soil and along the edges of forests. The leaves have at the base of the blade or along the leaf-stalk a swelling like a sac, generally double, "which opens on the under side of the leaf in the angles of the vein on both sides of the midrib". Food-bodies and extra-floral nectaries are absent. These pouches are inhabited by obligatory ants of the genera *Pheidole, Allomerus, Azteca* and *Crematogaster.* These Melastomaceae are especially represented by species of *Tococa, Majeta, Microphysca, Myrmidone* and *Calophrysa.* Melin comes to the conclusion that "we cannot explain the domatia in these plants inhabited by ants as an adaptation to ants that has originated through selection".

Myrmecophytes of Africa. Myrmecophytes in Africa are numerous. They represent trees, shrubs and lianas. Bailey (7) studied a number of them in the Congo and other parts of that continent. He found that *Vitex Staudtii,* which is inhabited by *Viticicola Tessmanni,* has lateral cavities or pits excavated in the woody part of the stele of stout dry stems and branches. Furthermore, there are in stout stems exit-holes resembling those of the lateral pits subtended by them. He suggests that it is likely that this characteristic in *Vitex Staudtii* may be due to an inherent tendency to form hollow stems and branches. It is not known whether the ants accelerate formation of the cavities throughout the center, as has been demonstrated by Fiebrig (49) with *Cecropia.* The pseudo gall-like structures made by *Viticicola* are histologically very complex.

In Africa there are a number of myrmecophilous species of

Cuviera. The ants are in elongated, spindle-shaped swellings in the branches. *Crematogaster africana* subsp. *Laurenti* var. *zeta* was found in *Cuviera angolensis,* and *C. impressiceps* var. *frontalis* in an unidentified species of the same genus. The myrmecodomatia of both species differ slightly from each other. Those of *Cuviera angolensis* are externally shorter and slimmer. They are not considered abnormalities produced by ants or by gall-forming organisms. They are hollow, localized hypertrophies of the branches in which ants are later found. These insects gain access to the interior of the swollen internodes through the circular apertures which are not as regularly formed as in *Vitex Staudtii;* their shape is apparently determined by the walls of the myrmecodomatia. Ants make their first excavations in the thin part of the stem which has also the widest surface. The myrmecodomatia of *Cuviera* possess inside oval pits and are commonly occupied by coccids of various sizes and ages.

Bailey observed that the walls of the myrmecodomatia "form a substratum for a more or less luxuriant growth of fungi". In the unidentified species of *Cuviera* he found dense growths of delicate, white hyphae.

Myrmecodomatia were found in a *Plectronia* species (Rubiac.) which resemble those of *Cuviera* and are inhabited by *Engramma Kohli.* Whereas in *Cuviera angolensis* the pith is homogeneous, that of *Plectronia* is heterogeneous. Two opposite parts of the swollen branch are much thinner than in that of the alternating part. The ants eat their way in the thin part, scarifying the inner surface. Along the margins of the irregularly formed openings heteroplasias originate. They resemble superficially those of *Cecropia.* According to Bailey, they consist of two distinct tissues, a central core of thick-walled, heavily pitted parenchyma, that is packed with starch as in the parenchyma of the normal and abnormal xylem, and an outer layer of thin-walled, isodiametric cells which are filled with an amber-colored, hyaline substance as occurs in the heteroplasias of *Cuviera.* The myrmecodomatia of *Plectronia Laurentii* have been especially studied by de Wildeman (189). *Barteria fistulosa* and *B. Dewevrei* (Flacourtiac.) were observed by Schumann (152), de Wildeman (189) and Bequaert (15). *B. Dewevrei* possesses hollow stems and branches, *B. fistulosa* has numerous hollow hypertrophied, deciduous branches that often have a fasciated

appearance.   The myrmecodomatia of *B. fistulosa* were found to be inhabited by *Crematogaster Buchneri* subsp. *biimpressa,* and those of *B. Dewevrei* by *C. africana* var. *Schumanni* and *Lecanium barteriae.*   It is of interest to note that de Wildeman (189) and Kohl (92) were able to find lateral pits in the myrmecodomatia of *B. fistulosa* in which coccids were observed.   In *B. Dewevrei* entrance apertures are usually found in that part of the wall of the myrmecodomatia which is much thinner than the other parts and is almost devoid of vessels.   The heteroplasias that partly fill them resemble those of *Cuviera*.

Interesting is a *Sarcocephalus* species (Rubiac.) described by Bequaert (15).   The myrmecodomatia that are inhabited by *Crematogaster africana* subsp. *Winkleri* var. *Fickendeyi* have circular openings closely below the node.   These domatia differ from those of many other species "in having four thin sides which alternate with four thick sides".   The heteroplasias have the appearance of those of *Plectronia Laurentii* and *Vitex Staudtii.*

Bequaert (15) and Schumann (152) describe among the African myrmecophytes *Schotia africana* (Leguminosac.), *Micaranga saccifera* and *M. Schweinfurthii* (Euphorbiac.) which have persistent pouch-like stipules in which ants have sometimes been observed. In *Cola* species the pouches at the base of the leaves have occasionally been found with ant-nests, especially in *C. Laurentii*.   In *Scaphopetalum Dewevrei* the leaves have at the base a fold in which numerous ants reside.   There are also peculiar pouches at the base of the leaf-blade of *S. Thonneri* native to the Cameroons and the Congo, 25 to 50 mm. in length, occasionally with ants.   *Epitaberna myrmoecia* (Apocynac.) from Cameroon has caulinary swellings in which the large *Pachysima aethiops* is found, a species much feared for its sting.   In *Uncaria africana* (Rubiac.) myrmecophilism is normal throughout a wide range of various parts of Africa.   The myrmecodomatia consist, according to Bequaert, "of the enlarged and hollow basal internodes of two opposite, lateral branches, the cavities in this pair of swellings communicating with the hollow, very slightly swollen node of the main branch".   The middle chamber is more or less club-shaped; it is dug further into the pith below them.   All species that were observed were inhabited by *Crematogaster excisa* subsp. *Anrei.*

There seems to be some nutritive value in the callus-heteroplasias

which grow from the margins of the openings. These tissues, as already stated, have been observed in different species. Their use may be complicated, due to the presence of coccids introduced into the myrmecodomatia and carefully tended by the ants as "milk cows" to which they devote considerable attention.

Myrmecophytes of Asia and the Indo-Malayan region. The number of species of myrmecophytes in this area is great. Among the most interesting are *Myrmecodia tuberosa* and *Hydnophytum montanum* (Rubiac.) which have been extensively studied by Treub (171, 172), Beccari (13), Rettig (136), Miehe (108) and others. They are often considered typical ant-plants and are native to the Malayan Archipelago and to Papuasia where they live as epiphytes in trees, often high in the crowns. They form thick, succulent stem-tubers that originate from the hypocotyle. The tubers are smooth in *Hydnophytum* and covered by sharp thorns in *Myrmecodia*. Those of the former are somewhat roundish and reach a diameter of 30 cm.; those of the latter attain a length of 0.5 m. Inside these tubers is a complicated and extensive system of galleries, at some places extending to the outside of the stem. They are populated by ants, especially by *Iridomyrmex myrmecodia* in Java and the Bismarck Islands; sometimes *Camponotus maculatus* has been observed. Both species of ants have been found on other species of plants, on fruits and in abandoned termite-nests. The sting of these ants is not so painful as that of other species. It was supposed by Beccari that these chambers were made by ants, but this has been contradicted by the researches of Treub who grew the plants from seeds with constant exclusion of ants. It was found that the tubers serve to store water which is necessary to these plants in their epiphytic mode of life. The walls of the chambers in the labyrinth are either smooth and light brown or blackish and covered by small wart-like particles. According to Miehe, the ants keep the pupae in the smooth rooms, while the excrement is put in the other rooms where a fungus also is found. Though the hyphae of this fungus are constantly kept short, it is not definitely known whether this species serves as food for the ants. Another fungus that has been observed has been identified as *Endosporum formicum*. It has been suggested that while the ants receive shelter in these tubers, the excrement of the insects serves as manure to the plant which needs it very much, due to its epiphytic existence and

rudimentary development of the root-system, which is not able to collect humus, as a few epiphytes are able to do, around the support-plant. Nitrification and formation of nitrates from the excrement of the ants has been observed in the chambers of the tubers. It has also been observed, as Treub states, that "Les fourmis défendent la plante contre des agresseurs"; and seeds have a few times been found to be disseminated by the ants. There may be therefore some foundation for symbiosis between plants and ants.

The genus *Endospermum* contains a number of species that are myrmecophilous and found in the eastern part of the Indo-Malayan Archipelago. Among them is *E. muluccanum,* mainly inhabited by *Camponotus quadriceps.* These insects work themselves into the pith of the stem. Some fungi have been observed but careful observations of Rant (134, 135) seem to indicate that they do not play a symbiotic rôle. Plants that were at first kept free from ants became infested by them as soon as there was a chance. Doctors van Leeuwen describe a myrmecophily of *E. formicarum* with the same species of ant in New Guinea. Large quantities of these ants live in the hollow parts of the stem and branches which run from the base to the top of the tree. The tunnels are not connected with that of the main trunk. Ants visit the extrafloral nectaries without bothering the caterpillars that eat the leaves, whereas in other cases, trees that are not inhabited by ants are sometimes not attacked by other insects at all. The hollow parts of the pith are connected with the outside world by small round openings in the bark. When trees are not attacked by ants the stem is filled by the tissue of the pith. This is removed by the ants in small pieces that are taken through the small openings in the bark. Doctors van Leeuwen found in these hollow parts two species of fungi, one resembling the species that was observed by Miehe in *Myrmecodia,* considered a species of *Septobasidium* by Neger. Spore formation has not been observed. The fungus probably grows on the excrement of the ants. This fungus growth is probably of no use to the insects. Whitish spheres have also been observed that have another construction; they too are composed of a mass of interwoven mycelium that may serve as food for the ants. In very young ant-colonies this fungus is already present. The ants, it is claimed, do not form any symbiosis with the tree, though they are apparently unable to live without the fungus.

Beccari has described a number of ant-plants from the Malayan region. He found many structures in the plants that he considered to be adapted for symbiosis between them and the ants. *Clerodendron fistulosum* and *C. myrmecophyllum* possess hollow chambers in the internodes of the branches in which ants have their nests. In the upper part of these internodes just below the leaves, two thin-walled layers in the parenchyma can be easily pierced by the ants and it is at these places that they force entrance into the hollow chambers. There is no doubt that the ants here find protection; it is, however, not known of what advantage they are to the plant. *C. fistulosum* is inhabited by *Camponotus clerodendri.* Schimper considers this member of the Verbenaceae a real ant-plant. Feeding bodies are absent, however. Miehe states that the lower surface of the leaves is covered by nectaries.

Though there are no ant-acacias native to the Indo-Malayan region, it is interesting to note that an *Acacia spadicigera,* introduced by Ridley from Jamaica and grown in the Botanical Garden at Singapore, was found to be taken possession of by some Asiatic ants in much the same way as ants do in tropical America.

In the genus *Macaranga* there are a number of species that are myrmecophilous, *e.g., M. triloba, M. Griffithiana, M. Hullettii* and *M. Hosei,* whereas some other species are not at all adapted for ant life. Ridley states that in *M. triloba* "we have the most perfect development of myrmecophily, and a true symbiosis". He proves that the guardianship of the ants is of the greatest value to the plant, which suffers from attacks of caterpillars when ants are absent. The hollow stem houses nests of *Crematogaster.* When the stems become tall the ants move their young to the higher nodes by perforating the septa of the nodes. Small, white, globular or elliptic bodies are found on the epidermis, which develop into "bud-bracts", oval, triangular, green bracts enclosing the buds. These globular structures are the food-bodies collected by the ants. They are taken in the mouth and carried to their nests in the hollow of the stem where they are fed to the larvae. It has been stated that somewhat similar bodies are found among certain Vitaceae, including species of *Leea, Vitis* and *Ampelopsis,* though they are not frequented by ants.

Raciborski (129) studied in Java a number of myrmecophilous plants, among which was *Pterospermum javanicum* (Sterculiac.).

In the axil of the petiole is a cup-like leaf that produces on its upper surface a large number of pearl-glands (Perldrüsen) that are gathered by the ants. These glands reach a length of about 0.3 mm. The food-bodies are absent on the outside of the cups. The life of these metamorphosed cup-like leaves is very short. The glands contain fatty bodies, albumen and polysaccharides. Raciborski states that he also found in the Botanical Garden of Buitenzorg, Java, *P. suberifolium, P. lanceaefolium* and *P. Heyneanum,* though not being visited by ants. The cup-shaped leaves were also lacking.

From the above examples it can be seen that myrmecophytes are extremely variable and that relationships exist which range from plain symbiosis to mild parasitism.

MYRMECOCHORES

Seeds and fruits are sometimes disseminated by ants. The number of such plant species is very large. The method of dissemination is different from that accomplished by other animals; some special attraction invites ants to carry the seeds, usually to their nests, certain bodies on the seeds, fruits *etc.* that offer to the ants oil, fat, sugar and starch.

Sernander (155) proposed the term "myrmekochores" and in connection with other terms like "anemochor", "hydrochor", *etc.,* he states: "Ich bilde analog als verbreitungsbiologischen Ausdruck das Adjektiv myrmekochor und die Substantive Myrmekochoren und Myrmekochorie".

Seeds are carried by ants to their nests for different purposes: (*a*) To eat off the plumule and radicle after germination. Such seeds are practically destroyed and are therefore of no use for distribution. (*b*) To use for construction of their nests, as is done by *Formica rufa.* (*c*) To use the important food source furnished by the elaiosome, an oily body, named by Sernander and considered an organ *sui generis.* Elaiosomes that have a loose construction, like those of *Chelidonium majus, Corydalis, Melampyrum* and *Veronica,* are entirely eaten by the insects. Among the various species these elaiosomes are found on different organs, seeds, fruits, sepals and the bases of spikelets.

Sernander, who studied mainly European myrmecochores, divides the plants into 14 types or groups. Representatives can also be found on other continents. These groups are:

1. Puschkinia-type.—The representatives belong to the Liliaceae. They do not possess an elaiosome. The testa has oil by which the ants are attracted. To this group belong *Allium ursinum, Ornithogalum nutans, O. Kotschyanum* and *Puschkinia scilloides* which is strongly attracted by *Formica exsecta.*

2. Viola odorata-type.—To this group belong a number of monocotyledonous and dicotyledonous families. The elaiosome can be easily removed. It is composed of the raphe, a part of the funicle that attaches the ovule to the wall of the ovary, and persistently adheres to the side of the seed. There is often an aril that surrounds the seeds. To this group belong *Luzula pilosa* whose seeds attract several species of ants, especially *Formica rufa* and *F. exsecta; Allium triquetrum, Chionodoxa luciliae* and *Lachenalia pendula* which is strongly myrmecochorous; *Chelidonium majus* which is also strongly myrmecochorous and attracts *Formica fusca; Corydalis fabacea, C. capnoides, C. nobilis* and others whose seeds are collected as soon as ripe by *Lasius niger* and *Formica rufifabacea.* Further, there are *Asarum europaeum* and *A. canadense.* Seeds of *Viola hirta, V. odorata* and other species are strongly desired by ants, also those of *Nemophila insignis.* To a considerable extent seeds are desired of *Veronica hederaefolia,* and those of *V. agrestis* and *V. panormitana* are collected to some extent by *Aphaenogaster barbara.*

3. Hepatica-type.—This is closely connected with the former group. The elaiosome is formed by the part of the pericarp. To this group belong *Theligonium cynocrambe* whose fruits are frequently distributed by *Lasius niger* and *Aphaenogaster barbara.* The fruits of *Hepatica triloba* are carried by several ant species. *Fumaria capreolata, F. officinalis* and related species are also of this type.

4. Parietaria lusitanica-type.—This group possesses a small swelling toward the base of the perianth and receptacle. The seeds of *Parietaria lusitanica* are distributed by *Formica fusca, Aphaenogaster barbara* and others. Here belongs also *Polygonum capitatum.*

5. Ajuga-type.—In this group we find the elaiosome belonging to a part of the receptacle that is connected with the fruit. Most species of this group belong to the Labiatae and Boraginaceae, *e.g., Borago officinalis, Myosotis sparsiflora, Pulmonaria mollissima, P.*

officinalis, Ajuga Chamaepitys, Lamium album and *Rosmarinus officinalis* whose seeds are especially collected by *Aphaenogaster structur, A. barbara* and *Tetramorium caespitum.*

6. Aremonia-type.—The flower-axis of the fruit forms a fleshy structure that is liked by ants. To this group belong *Thesium alpinum* and *Aremonia agrimonoides.*

7. Carex digitata-type.—An oily body develops at the base of the utricle. It is found on *Carex digitata* and is collected by *Formica rufa.* Plants are frequently found near the nests of this ant species. Here belong also *C. ericetorum, C. montana* and others.

8. Melica nutans-type.—In this group the elaiosome is found toward the base of the spikelet. This part of *Melica nutans* is very much liked by several species of the genus *Formica* and *Myrmica.*

9. Euphorbia-type.—Here is found a caruncle formed by the enlarged lips of the appendages near the micropyle. According to Sernander, this type merges into the *Viola odorata*-type. To this group belong *Euphorbia peplus, E. peploides, E. Lathyrus, E. segetalis* and others. In *E. helioscopia* the caruncle is not changed into an elaiosome. The seeds are very much appreciated by species of *Formica rufibarbis, Lasius niger* and *Aphaenogaster testaceo-pilosa.* To this group belong also *Mercurialis annua, M. perennis, Viola arvensis* and others.

10. Polygala-type.—These contain clearly two elaiosomes, a large one at the base and a small one at the top. To this group belong *Polygala monspeliaca* and *P. vulgaris.*

11. Amberboa-type.—This group is characterized by the short spiny pappus. In comparison with the *Trichera*-type it has a similar oil-body. Here belong *Centaurea Cyanus, C. Scabiosa* and some others.

12. Galactites-type.—The base of the style has formed an elaiosome. Here belongs *Carduus pycnocephalus* which is strongly myrmecochorous. Its seeds are transported by *Aphaenogaster barbara, Formica fusca* and *Lasius niger.*

13. Trichera-type.—The base of the fruit is surrounded by a circular undulate elaiosome. The fruits of *Trichera arvensis* are much appreciated by *Formica rufa, F. rufibarbis* and *Lasius fuliginosis.* Here belongs also *T. orientalis* which is collected by *Formica rufa.*

14. Triodia-type.—There are two oily bodies formed toward the

base of the spikelet. There is a weak myrmecochory. To this group belongs *Triodia decumbens* which is collected by *Formica rufa*.

Sernander studied the number of seeds that were carried by ants to their nests and found that *Aphaenogaster* carried 216 seeds in 2 hours; and *Formica rufa,* 366 seeds in 19 hours, among them being 157 of *Melica,* 69 of *Melampyrum,* 28 of *Hepatica triloba,* 25 of *Carex digitata* and 21 of *Luzula pilosa.* The seeds were carried for various distances as far as 75 meters.

Time of ripening of seeds or fruits among myrmecochorous species much affects their distribution by ants. Seeds of many ripen early in summer and have therefore much chance of being distributed, while some ripen late in autumn when the ants prepare to retire into their winter quarters.

Seed distribution by ants has also been studied to some extent in other continents, by Bews (17) in Africa, by Lock (97) and Doctors van Leeuwen (39) in Asia, Lincecum (95) in North America and Ulbrich (174, 175) in South America.

It is of interest to note that many myrmecochores are related to non-myrmecochores, some being anemochores forming no elaiosomes. Among the first group are *Luzula pilosa, Anemone nemorosa, A. ranunculoides, Potentilla alba, P. reptans, Primula acaulis* and *Myosotis sparsiflora;* to the second group or non-myrmecochores belong *Luzula campestris, Anemone silvestris, Potentilla rupestris, P. recta, Primula elatior, P. officinalis* and *Myosotis silvatica.*

The seeds of a number of plant species that grow against walls have been distributed by ants. The number of these species is considerable. First among them one encounters *Chelidonium majus,* then also *Lamium album, Glechoma hederacea, Veronica hederifolia, Sedum acre, Cerastium nivale, Stellaria media, Arenaria serpyllifolia, Viola odorata, V. canina* and *Melandrium album.* Of the 90 species of such plants there are 60 that are myrmecochorous.

To this group belong also the ant-gardens or ant-epiphytes that have been studied extensively by Ule (176) in the region of the Amazon. These ant-gardens are often found high in the trees of the tropical forest and are composed of species that belong to different families. Among these are species of *Peperomia* and *Codonanthe.* There is also a considerable number of Bromeliaceae.

Their seeds are carried to the high parts of the stems or branches by ants. In the ant-gardens are species not otherwise among epiphytes. Ule observed how ants sucked juice from berries of *Nidularium* and *Portea,* which were soon afterward carried to their nests in the trees. Bromeliads and some species of *Anthurium* occupy the center of the nests, whereas species of Gesneriaceae and of *Ficus* are found toward the outside. *Peperomia* species hang usually with their long shoots downward. The ants sow and cultivate these species, many of which could otherwise have no existence. The mutual relation between ants and plants Ule does not consider as a protection-symbiosis (Schutz-symbiose) but more as a symbiosis for space (Raumsymbiose). Ant-gardens often reach gigantic dimensions in the crowns of trees.

Also among the occasional epiphytes are species that have been distributed by ants (181). Beyer (18) found that among 246 species there were 60 whose seeds were distributed by ants, among these being *Chelidonium majus, Viola odorata, Melandrium album, Stellaria media, Lamium album, Arenaria serpyllifolia.*

Phylogenetically, myrmecochores have been considered a young group. They may have originated in some cases from anemochorous species. To these may belong the *Centaurea-, Fedia-, Knautia-, Triodia-* and *Polygala*-types.

ANTS AND EXTRAFLORAL NECTARIES

Extrafloral nectaries are those outside the flower. They are usually on the petiole, at the top, toward the middle, or near the base, or they may be on the leaf-blade. The typical sugar-excreting nectary is only in the flower, as originally defined by Linnaeus in his Philosophia Botanica, 1751, as "nectarium, pars mellifera flori propria".

Many investigations on extrafloral nectaries have been conducted by Delpino (36, 37) who supposes "that the chief function of these nectaries is to place the ants, wasps and polistes in the position of sentries and guards, to prevent the tender parts of the plant from being destroyed by larvae". He holds that where ants are present larvae will be devoured. In other papers Delpino proposed that all plants having extrafloral nectaries be placed among the myrmecophytes. This view, however, could not be accepted, though it has been carefully considered by Schimper (148) and Kerner von

Marilaun (87). The most classical examples are *Vicia sepium* and *Prunus* species. There are many objections. It is true that ants are often found on some of these plants, collecting the sweet substance from these glands. On the other hand, numerous species having extrafloral nectaries are never visited at all by ants, as has been shown by extensive studies of Nieuwenhuis von Uxküll-Guldenbrandt (119), conducted in Buitenzorg, Java. She comes to the conclusion, as far as these particular species are concerned, that structure, form and position of the extrafloral nectaries are beneficial and in some instances could not possibly be related to ant protection. It was also found that location and appearance of the nectaries on the plant is often impractical from a standpoint of myrmecophily. There are also a number of plant species that are provided with domatia, food-bodies, extrafloral nectaries, protostomata, *etc.,* that are seldom or not at all touched by ants.

Recently Springensguth (162) gave a general account of these nectaries in relation to insect visits. He lists 509 different species of insects that were found on the extrafloral nectar glands of several species of plants. This list proves that ants form $\frac{1}{8}$ of the insect visitors. Use of these glands is difficult to understand and it has been suggested that the plant can do as well without them. There is here hardly any direct symbiosis between the plant and ants or other insects.

Of the very few examples relating to ant-protection those of von Wettstein (186) may be regarded as most conclusive and convincing. He observed that in *Centaurea alpina, Jurinea mollis* and *Serratula lycopifolia* there exists some kind of protective relationship with ants. The simple extrafloral nectaries on the bracts of the involucrum are readily visited by ants. When ants are artificially kept from these inflorescences the flowers are most liable to be attacked by certain beetles.

THE FUNGUS-GROWING HABIT AMONG ANTS AND TERMITES

The growing of fungi by ants and termites for their own benefit has been studied since the end of the eighteenth century. The systematic position, especially of the termite-fungi, has been settled only in special cases, and there is much need of classification. Fungi grown by these insects in their nests show morphological peculiarities not found when cultivated under other conditions, and

one wonders whether these differences are not induced by chemical excretions of the insects, among the ants, for example, by different formic acid compounds that are able to make these changes. As to the occurrence of these fungi, the statement recently given by Heim (66) that there is a typical fungus-flora in the termite-nests is very significant, and it seems also to be the case with some fungi found only in ant-nests.

A number of suggestions exist, trying to explain the origin of the highly specialized nature of the attine ants in relation to their remarkable ability of raising fungi. Von Ihering (80) presumes that the fungus-farming and leaf-cutting habits originated from an earlier seed-storing habit. Forel (53) is of the opinion that in the early stage of development of the biocoenose, the fungi might have been grown on excrement of insects. Wheeler (188) supposes this to be the origin of the fungus-growing habit of termites as well. He states further; "the method employed by the *Atta*-queens in manuring their incipient fungus-gardens suggests that the food plant may have been originally grown on fecal substances".

ANT-FUNGI

A considerable number of ant species raise fungi that are used by them as food. These insects are not found in warm countries only, as is often supposed. A number are also found in temperate regions. *Lasius fuliginosus,* often in Central Europe in hollow tree-trunks, regularly has the fungus *Septosporium myrmecophilum* along the walls of its nests. This symbiotic condition was at first observed by Fresenius (54) and was later studied in detail by Lagerheim (93). He came to the conclusion that the mycelium was used as a kind of building material in the nests. Neger (118) observed much later that the mycelia secreted small drops of liquid that were readily taken up by the ants.

Those who have travelled in tropical countries can hardly be unacquainted with those ants that cut cylindrical parts from leaves, often from high in the trees. These parts of the cut leaves are carried to their nests. As early as 1863 Bates (11) mentions these leaf-cutting ants in his "Naturalist on the River Amazon". He questions the use of the large amount of leaves harvested by stating: "It has not hitherto been shown satisfactorily to what use it applies the leaves". Belt (14) in 1874 calls these insects "foraging ants"

in his "Naturalist of Nicaragua" and states: "This mass which I have called ant-food, proved on examination to be composed of minutely subdivided pieces of leaves, withered to a brown colour, and overgrown and lightly connected together by a minute white fungus, that ramified in every direction throughout it". Belt found this fungus not only in every chamber he opened but also in those of the nest of a distinct species that generally comes out at night. He was convinced that the ants do not eat the leaves. He was able to observe refuse particles of the leaves that had been exhausted as a substrata for the fungus and were left to be used by other insects. Also Müller (116), in a letter to Charles Darwin which was published in Nature, mentions that he held the same view, that the ants feed upon the fungus growing in the leaves which they carried to their nests.

These interesting observations stimulated Möller (114) to make a series of studies of fungus-gardens, which he called originally Pilzgärten, especially those of certain South American ant species. In the nests of species of *Atta,* the true parasol or leaf-cutting ants, are loose, soft, gray, flaky, hollow parts, having the appearance of a coarse bathing-sponge. These gardens are never found exposed. They belong to species that have their nests in trees, like *Atta discigera,* as well as to species that have their nests in the ground. Mushroom-gardens were found that had a total length of 1.5 meters. The hollow parts are irregular. *Atta hystrix* and *A. coronata* were found to be more common in forests; *A. discigera* occurs generally near human habitations. The ants not only collect circular sections of the leaves but also use other plant material. Mycelia are in every particle of the nest and belong always to the same fungus species. The hyphae show several cells and a large number of anastomosae (Fadenbrücken). At the ends of the hyphae occur typical roundish tips, kohlrabi-like in shape, bromatia (Kohlrabihäufchen). They are 10 to 24 microns in diameter. They occur in dense groups toward the surface though not in the inner part of the mycelium mass. These roundish bromatia are alike for all species of *Atta,* and are eaten by them. They are removed by their strong jaws, which requires no special strength. Aided by the feet of their front legs, with the slightly opened jaw the food is turned and pressed in all directions during eating, and the mouth-parts suck the kohlrabi-like body which finally disappears through the mouth.

During this manipulation the ends of the antennae are in constant touch with the food. The fungus-gardens are a few centimeters in diameter and are irregular in shape. The nest of the much lower genus *Cyphomyrmex* has small crude fungus chambers. They collect excrement of caterpillars on which is grown a mycelium that forms well developed bromatia or food-bodies. The nests of *Sericomyrmex, Mycetosoritis, Apterostigma* and *Trachymyrmex* are more regular and form pendant mushroom-gardens on remnants of vegetable origin and insect excrement.

When disturbed, even the smallest parts of the fungus-gardens are brought to safety. Gardens that have been exposed are soon covered by the ants. Experiments have shown that the fungus-gardens are highly prized, by the protection given them by the ants. Species of other genera besides *Atta, e.g., Apterostigma* and *Cyphomyrmex,* grow and eat fungi, though they apparently do not cut the roundish sections from the leaves. Other characteristics of the fungus are described by Möller in detail, especially under laboratory conditions. Often they give rise to a form of conidia, and other formations are observed on the mycelium. Under the influence of the ants no formation of any free air-mycelium or any typical fructification occurs. Under a number of conditions large fruit bodies were observed that proved that the ant-fungus belongs to the Agaricaceae; it was identified as belonging to the group Lepiotae and was given the name of *Rozites gongylophora.* Also the fungus that is grown by the hairy ants, *Apterostigma Wasmanni, A. pilosum* and others, is probably the same species. The kohlrabi-like food-bodies in the nests of *A. Wasmanni* are less pronounced though more conspicuous than those of *Cyphomyrmex strigatum,* and are least developed in nests of *C. auritus* and *C. pilosum.*

The bromatia or feeding-bodies at the ends of hyphae have to be considered as distinct morphological entities.

Formation of new colonies among the attas who transfer the fungus-culture from the original mother-nest, has been studied by von Ihering (80), Goeldi (58, 59) and Huber (79). Apparently the dilated fertilized female of *Atta sexdens* often starts a colony alone; she digs a burrow in the soil, and at a depth of 20 to 30 cm. a chamber is formed in which eggs are soon deposited. Near the eggs a mass of loose white matter is found about one to two mm. in

extent which forms the earliest beginnings of the mushroom-garden. Ihering found that every *Atta*-queen, when leaving her original or parental nest, carries a loose pellet of debris in her infra-buccal pocket containing some hyphae of the fungus-garden. This was confirmed by Huber. At first the queen does not eat the fungus, but a part of her own eggs, as do the first larvae. Soon after hatching, the workers manure the garden with fecal excrement and feed the larvae with some of the queen's eggs, while they themselves feed on the kohlrabi-bodies that develop on the fungus. Later these bromatia become so abundant that they can be fed to the larvae.

Goetsch (60–61b) and Goetsch and Stoppel (61c) deny the statement that *Rozites gongylophora* is the only species used as food. They isolated from nests of *Atta sexdens* from Brasil the following fungi: *Hypomyces ipomae, Fusarium oxysporum, F. oxysporum aurantiacum, F. angustum, F. equiseti, Verticillium candidum* and *Clonostachys araucariae*. From nests of *Acromyrmex* were gathered *Actinomucor repens, Mucor racemosus, Moniolopsis Aderholdii, Trichoderma* sp., *Penicillium* sp. and *Rhizopus nigricans*. *Actinomucor repens* was always present. Material that was moistened or saturated with saliva of the ants resulted in pure cultures. *Fusarium* grew rapidly, *Hypomyces ipomae* became flaky without forming conidia, whereas *Penicillium verticillatum, Rhizopus* and others were entirely suppressed. Under these conditions pure cultures originated and the "weeds" were eliminated. The "domestic" fungi developed profusely without giving rise to spores. Under pH 4.5 the fungus garden of *Actinomucor* became overgrown with *Hypomyces,* the cultures became pure at pH 6–7. Besides acidity certain enzymes in the saliva of *Acromyrmex* are considered to be of importance in cultivation of the ant-fungi. It was found that the saliva of other ant species was no more effective than that of humans. Hungry *Acromyrmex* ate *Fusarium* and *Hypomyces* to such an extent that no mycelium was left.

Among the fungus-growing ants in North America Wheeler (188) mentions *Atta texana, A. versicolor chisosensis, A. tirrifex, A. Hartmanni* and *Cyphomyrmex rimosus* var. *comalensis* from Texas; *A. septentrionalis* is distributed from Texas to Florida, North Carolina, New Jersey and the District of Columbia; *A. jamaicensis* occurs in the West Indies; *A. Smithi* is from Cuba;

Cyphomyrmex rimosus is found from Central America to Texas; *C. rimosus* var. *minutus* lives in the West Indies and Florida; *C. flavidus* occurs in Mexico, *C. Wheeleri* in Texas and California, and *Myrmicocrypta Brittoni* was found in Porto Rico.

The mycelium of *Atta septentrionalis,* the most widely distributed species, has a bluish tint, resembling that of *Penicillium glaucum.* The gongylidia are pear-shaped, 4.5 microns in length and 3.6 microns wide. They are grouped in compact masses 0.4 mm. to 0.5 mm. wide.

Wheeler has described the fungus in nests of *Cyphomyrmex rimosus* var. *comalensis* as *Tyridomyces formicarum,* provisionally grouping this peculiar species among the Exoascaceae. Furthermore, Farquharson (48) states that certain Nigerian Cremato-gasters are fungus-farmers. Donisthorpe (42), in his work "British Ants", presents the view that *Cladosporium myrmecophilum* is used as food by *Lasius fuliginosus,* whereas *L. umbratus* cultivates *Hormiscium pithyophilum* var. *myrmecophilum.* These fungi occur as "pure cultures" in the nests. There is, he states, a particular fungus species for each ant species which suggests that other Formicidae are fungus-growers and not only Attii alone.

TERMITE-FUNGI

Fungi were found in the nests of termites several decades ago. Koenig (90) in 1779 was among the first who found them in nests of *Termes bellicosus* "an den Wänden der Magazine ein Art Schimmel (mucor stipulatus capsulis globosis compositis niveus) welche vielleicht den jungen als Nährung dient." Fungus-raising proved later to be far more widely distributed among termites than among the ants. It was shortly afterward, in 1781, that Smeathman (159) observed that some species of termites in tropical Africa grew in certain chambers a fungus that was used by them as food; he calls these gardens "nurseries", and states: "There is one remarkable circumstance attending the nurseries. They are always slightly overgrown with mould, and plentifully sprinkled with small white globules about the size of a small pin's head". These observations were later confirmed by others, for example, by Savage (144) in 1850 who considered the fungus as a kind of *Mucor.* Later Penzig and Saccardo (122) described a form of *Xylaria* obtained from termite-nests in Java. Later it was found by others that species of *Xylaria* were frequently present but are to be considered as "weeds".

Berkeley (16) describes the edible *Agaricus termitigina* from India, which is probably identical with *Lentinus cartilagineus* also found in the nests of white ants.   In 1870 Cesati wrote about *Tricholoma subgambosum,* probably a termite-agaric from Borneo. Much work has been done by Holtermann (77, 78) who studies termite-fungi in Ceylon, Java, Singapore and Borneo.   He placed a termite-comb in a glass dish where it developed a large amount of mycelia, forming white strands as thick as a finger, which he described as *Pluteus rajap,* but which, according to Petch (123), was the mycelium of a *Xylaria.*   Later Höhnel (76) studied termite-nests in Java and was able to confirm much of the work of others in Ceylon.   He not only described *Xylaria* but mentioned the pyrenomycete *Neoskofitzia termitum.*

Karaiwaiew (86) gives a description of white, roundish masses in a termite-comb from Buitenzorg, Java.   Doflein (41) made similar observations on termite-nests in Ceylon.   He noted white spheres and found that when the comb was placed under a bell-jar, it formed several long cylindrical bodies of fructifications that were, according to Holtermann, incomplete *Xylaria*-stromata.

From South America Hennings (71) describes *Pluteus termitus* which he found in Brasil, and Theissen (168) mentions *Xylaria nigripes* in termite-nests from the same country.

Trägardh (170) in 1904 mentioned the fungus-growing termites in Sudan, among which *Termes natalensis* has combs whose surfaces are covered with a fine feltwork of mycelium.   Some interesting work on the subject has been done by Jumelle and Perrier de la Bathie (84) in Madagascar where the combs of *Termes Perrieri* carry conidial spheres having a diameter of more than 1 mm. and much the same appearance as those in other countries.   They originate from a mycelium that runs through and along the comb. Near the base of the comb the mycelium develops numerous small protuberances that are regarded as supports.   When the insects abandon the comb of the nests, the scanty layer of mycelium around the comb soon gives rise to a large number of hyphae growing into a thick felt which is called by the authors "forme evahissante". Within a few days sclerotia of different size and shape may be seen, which remain sterile during the dry season but on which *Xylaria termitum* develops in the wet season, closely resembling *X. nigripes.* Sjöstedt (157) observed in Cameroon that workers of *Termes*

Lilljebergii cut small round parts from the leaves, which they carried to their nests in much the same way as leaf-cutting ants do.

Petch (123) concludes that a white "conidial" sphere and *Agaricus* spp. develop on the comb within the inhabited nests and that *Xylaria* spp., including *Sclerotium,* and *Peziza epispartia* develop in the comb after the termites abandon their nests, or when a comb is placed for some time under a bell-jar. On the other hand, he considers *Podaxon* spp. and *Neoskofitzia termitum* as probably adventitious. He mentions the interesting facts that the agaric undoubtedly arises from the termite-comb, not merely from the soil in the neighborhood of the nest, that it grows from the comb while the nest is inhabited, and that it has never been found in any other situation. There is a long list of synonyms of a relatively few termite-fungi which proves sufficiently the difficulty of identifying the species. One of the principal species is *Collybia albuminosa* which is also the principal edible mushroom among the Cingalese.

Bose (22) and Annandale (3) have enriched our knowledge of fungi from termite-nests in Barkuda, where at least three different species are known to cultivate fungi, *i.e., Microtermes anandi, Eurytermes Assmuthi, Adontotermes obesus* and *A. obesus* var. *oculatus.* According to Annandale, it is possible to demonstrate an evolutionary series in the structure of the fungus-combs among these termite species, with the combs of *M. anandi* at the base and those of the typical form of *A. obesus* at the top, those of *E. Assmuthi* being a little more advanced in structure than those of *Microtermes,* and those of the variety *A. obesus* less advanced than those of forma *typica* of the same species. In the nests of *A. obesus* and its variety *oculatus* he found that there were only workers in the garden-chambers and fewer soldiers. If a mound of this species is opened in February the upper garden-chambers above ground are empty; in April and May all garden-chambers in the mound are empty and entirely clean. When the colony has died out, dry and shriveled remains of the garden are seen in abundance. The termites feed not only on fungi but also on leaves, wood and other dead organic matter. Bose found in these nests *Xylaria nigripes;* in no case were stromata produced from combs *in situ* within the mounds. He found that the termites regularly cultivate *Collybia albuminosa* for food; they eat the mycelium and "spheres", whereas it is supposed that they "weed out" the growth produced by *Xylaria.*

Annandale states that during the dry season and especially when weather is hot as well as dry the workers remove the gardens bodily underground.

DIFFERENT HYPOTHESES REGARDING MYRMECOPHILY

A number of hypotheses try to explain the cause and purpose of myrmecophily. Some are old, others of recent origin. Inasmuch as many are based upon opinions, their rise or decline is dependent upon their value in connection with confirmation of actual facts.

It was Spruce (164) in 1869 who supposed that all unusual structures of ant-plants originated by the action of ants, without which, leaves, branches, *etc.*, would revert to their original form. Beccari (13) in 1884, in his work on myrmecophytes of Malaysia, expresses the same views, thus in the Lamarckian sense admitting an inheritance of acquired characteristics. In a statement to Charles Darwin he says: "The ants cannot be said to be useful to the plants, anymore than fleas and lice are to animals; and the plants have to accommodate to their parasites as best as they may". Belt (14) in 1874, studying myrmecophilous acacias, concluded that ants "form a most efficient standing army for the plant, which prevents not only the mammalia from browsing on the leaves, but delivers it from the attacks of a much more dangerous enemy—the leaf-cutting ants". The ants receive secure housing from the plant and are supplied with a sufficient amount of food. He also explains the value of extrafloral nectar glands as serving to attract insects, especially ants, protecting flower-buds and other parts from destroying-insects and other animals. This view was also dealt with by Delpino (36, 37) and to a considerable extent by Müller (117) and Schimper (148). These views were later doubted by Möller (114), Ule (176), Rettig (136), Fiebrig (49, 50) and especially by Nieuwenhuis von Uxküll-Goldenbrandt (119) in Java.

According to Bailey (6, 7, 8), the frequent occurrence of the same peculiar structural modification in plants not inhabited or visited by ants, "is as serious a stumbling block in the way of the Spruce-Beccari hypothesis as it is in that of Belt-Delpino".

Buscalioni and Huber (28), who studied ant life in the Amazon region, came to the conclusion that plants which are inhabited by ants come from regions that are or were once periodically inundated. It occurs to them that there must be some relationship

between myrmecophily and inundation of the area. If a genus is represented by a myrmecophilous as well as an ant-free species in inundated and non-inundated areas, usually the land forms are without ant-nests and those from the flooded districts are myrmecophilous. The authors claim to prove this by mentioning *Cecropia* species (except *C. adenopus*); furthermore, *Triplaris* has two myrmecophilous species in the flooded districts and ant-free species in the mountain forests, among which is *T. Gardneriana*. Myrmecophilous species on non-inundated land may be derived from inundated areas, as *Cecropia adenopus,* or may grow in places that were regularly flooded in times long past, *e.g.,* species of *Tococa.* Ant-plants from strongly flooded areas are usually trees, *e.g., Cecropia* and *Triplaris;* those of places slightly flooded may also be shrubs, *e.g., Cordia* spp. and a number of species of the Melastomaceae. One is therefore supposed to think that the ants went to these "tree-islands" for protection against floods, building their nests in safety. Fiebrig (49, 50) also suggests this possibility and mentions that of 70 species of the genus *Azteca* all are dendrophilous.

The existence of symbiosis between such plants as *Cecropia* or *Acacia* and ants has at times been doubted, as has already been mentioned. Some regard it as parasitism at one extreme, others as a kind of symbiosis at the other extreme where the plants are the givers and the ants the takers without incurring damage to the plant.

The relation between fungus-growing ants and termites and the fungi that are cultivated is a far more striking example of symbiosis. While the insects eat only a certain part of the fungus, the fungi, on the other hand, receive a proper amount of shelter in the form of darkness and nourishment, and special attention or "looking-after". Moreover, it occurs that at least some species of these fungi are especially confined to these nests, among these being *Rhozites gongylophora* associated with certain ants, and those that are found only in the nests of termites. It was Heim (66) who quite recently stated: "Il existe une mycoflore termitophile comme il existe une mycoflore lignatite, ou coprophile".

Also the relations found in myrmecochory are apparently of a symbiotic nature. The elaiosome of the seed of certain plant species is such a delicacy to a number of ants, and dissemination of the seed by the ants when they carry them to their nests is of such value to the plants, that usefulness to both symbionts is plain.

In the entire field of symbiosis between ants or termites and plants, it is difficult to understand that we have to do with an adapted or acquired symbiosis underlaid by a teleological approach rather than with a symbiosis where during the passing of time, under evolutionary processes, the plants and insects were incidentally brought together to their more or less mutual advantage, a relationship that may vary in a different environment or that may even become inactive to one of the members.

It may have been possible that some of the uninhabited plant species that are apparently adapted to housing ants had species of insects in ages past which have become extinct, and the present living species of ants cannot adapt themselves for some reason to these particular antless plants; or, after all, they may never have been inhabited by ants in the past.

SUMMARY

An ecological relationship exists between plants and some species of ants and termites, insects that have reached a relatively high degree of "mental" and social organization.

Myrmecophytes are plants that harbor ants. They are myrmecotropic when they provide food, myrmecodomic when they give shelter only, and those that provide food as well as shelter are termed myrmecoxenic. Some typical ant-plants are *Cecropia peltata, Acacia cornigera, Myrmecodia tuberosa* and *Hydnophytum montanum*. Belt's food-bodies at the apex of the lower pairs of leaflets of *Acacia* are supposed to be of glandular origin and may be related to hydathodes. Also the Müllerian bodies of *Cecropia* are considered by some to be of glandular origin. These bodies form an important source of nourishment to those ant species that inhabit these particular plants. Some recent investigators consider them a kind of adaptation to ants; outside of this their botanical value would be entirely unknown. *Macaranga triloba* has been stated to "have the most perfect development of myrmecophily, and true symbiosis". There are other instances where instead of symbiosis a kind of parasitism is considered to exist, or at least the plant is the giver and the ants the takers who give neither protection nor serious damage to the plant.

Myrmecochores are plants having seeds or other floral parts provided with tissues, such as elaiosomes, that are used by ants as food.

The seeds are carried to the ant-nests and are thus aided in dissemination. These species are numerous, and to them belong *Chelidonium majus, Viola odorata, Veronica hederaefolia, Hepatica triloba, Euphorbia peplus.* These myrmecochores can be divided into at least 14 different groups, some of which originated from anemochores.

It is very doubtful whether extrafloral nectaries are of any use to the plant as far as symbiosis with ants is concerned. *Centaurea alpina, Jurinea mollis* and *Serratula lycopifolia* may offer some of the few exceptions.

Fungus-growing among ants is especially common among species of *Atta,* or leaf-cutting ants. They grow certain fungi in their nests as food. At the same time, the fungus receives care and nourishment through the ants, thus suggesting a distinct symbiotic condition. Fungus-growing among termites is even more widely spread than among ants.

It is not possible to accept the term "myrmecophily" in the old orthodox concept where it is understood that there is a distinct symbiotic relationship between plant and ants. This idea has to be considered in a much looser way where widespread variations occur and where the members may live in true symbiosis, whereas in other instances they almost reach a mild parasitism. There is, however, no reason that the word "myrmecophily" be eliminated for a host of other terms that would only make confusion. Each instance has to be considered under the same general term.

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PARTHENOCARPY: NATURAL AND ARTIFICIAL

FELIX G. GUSTAFSON
University of Michigan

INTRODUCTION

That some plants produce fruits without seeds is a fact observed and recorded by the ancients, according to Sturtevant in 1890. These observers had their pet theories on how to produce seedless fruits, such as "fruits of all kinds may be grown without seeds by reversing the cion—rooting the top end of the cions", or by removing the pith from grapevines. While there were many others, we owe especially much of the early careful observations and scientific attempts to produce seedless fruits to Gärtner (44), Munson (103, 104), Müller-Thurgau (101, 102), Waite (154, 155), Noll (110), Ewert (31, 32, 33, 34), Winkler (159, 160) and Fitting (35, 36, 37). These men thought it possible to obtain varieties of apples, pears, cherries, plums, peaches, grapes and other fruits without seeds. They were interested in this matter for many reasons but mainly because seedless fruits were thought to be better and also because many varieties are self sterile, necessitating the planting of more than one variety in an orchard to insure a profitable crop.

In 1902 Noll (110), recognizing the similarity between seedlessness in fruits and parthenogenesis, introduced the term "Parthenokarpie" to denote the seedless condition, and this term is now in general use, though sometimes the Germans use "Jungfernfruchtbildung" or "Jungfernfruchtigkeit". Although Noll considered that perhaps all seedless fruits should be included under this term, whatever the cause of seedlessness, he restricted it to denote fruits produced without pollination or other stimulation. Winkler (159) in 1908 defined parthenocarpy "as the production of fruits without or with empty seeds", and he differentiated between stimulative parthenocarpy in which a fruit is produced only after pollination or other stimulation, and vegetative parthenocarpy in which no pollination or other stimulation is necessary. Fitting used the term "autonomous" for vegetative and "aitionomous" for stimulative parthenocarpy. At present parthenocarpy seems to be used to denote production of fruits without fertilization; and to denote seedless fruits produced as a result of fertilization but with early

abortion of the embryo, Stout (138) has used the designation "stenospermocarpy". In practice it is, however, very difficult to distinguish between seedless fruits produced as a result of fertilization followed by very early abortion and those produced without fertilization. In both, the ovules may be very minute, or an empty, partly developed seed may result. Therefore, in this review parthenocarpy will be used to designate any seedless fruit unless it is definitely known, as in some varieties of grapes, that fertilization and abortion have taken place. Also will be included seeded fruits known to have had the embryo produced in other ways than by fertilization of the egg, as in mangosteen and some oranges.

In order that the review may not become too long no papers before 1890 are included. The reader interested in earlier work may refer to Sturtevant's excellent paper, "Seedless fruits", which was publishd in 1890. However, while no papers as a whole are reviewed, references will here and there be made to work done before this date, and Sturtevant will be cited as the authority rather than the original author whose paper has not been read by the reviewer.

The review will be divided into two parts. Part one will deal with natural parthenocarpy in which the seedless condition may or may not have been aided by man taking advantage of a natural tendency in a plant to produce fruits without fertilization, and in the second part will be reviewed those papers in which parthenocarpy was induced by chemicals. While some papers under section one may deal with fruits produced artificially, this was almost always done by producing more favorable conditions for natural parthenocarpy to express itself, and there can be no real separation of parthenocarpy thus furthered by man from that which took place without the aid of man. In the second part, on the other hand, will be included those papers dealing with plants that normally do not produce fruits unless fertilization takes place, and here we are very definitely dealing with induced or artificial parthenocarpy.

NATURAL PARTHENOCARPY

Acer. Beketovskie and Beketovskie (8) report observing parthenocarpy in *Acer Negundo* and in its golden-leaved variety *odessanum.* It was more frequent in the typical form than in the variety. They also report that parthenocarpy was stimulated by chalk dust, *Lycopodium* spores and by pollen of *Corylus avellana.*

The experiments were not too carefully controlled, however. In some instances they found one-half of the achene seeded while the other was empty.

For several seasons the writer has observed numerous empty fruits of *Acer saccharum* near his home. In the fall of 1940 several hundred fruits from different parts of Ann Arbor were examined. It was found that at least 50% were empty, and as on careful examination the ovules could be seen only as dark specks, this is undoubtedly a case of parthenocarpy. Most achenes had only one of the fruits empty.

Aethionema grandiflorum. At Freiburg, Hildebrand (69) had one specimen of this species growing in the Botanic Garden with other species of the same genus. It produced fruits sparingly, but during a period of at least eight years he never obtained any seeds.

Ananas sativus (Pineapple). The pineapple of commerce is seedless, or if seeded fruits are formed they are so rare that most of us never see a seeded fruit. In cytological studies of two races, Tanguel and Baños, Heilborn (63) found that not all pineapples have poor pollen; in fact, most have perfectly normal pollen. In a private communication Sideris (133) states: "The pineapple flower produces a parthenocarpic fruit as long as it is either self-pollinated or not pollinated at all. If, however, the pollen of a different variety is applied to the stigma of the Smooth Cayenne or other varieties, then seeds develop. The parthenocarpy in the pineapple is due to self-sterility or self-incompatibility, but not to the functional potentialities of either the pollen or the ovules". Collins (23) adds: "We have observed several distinct mutations in the pineapple which removed the incompatibility and permits self setting of seeds. A few wild species of *Ananas* set self seeds regularly and the fruits of these are nearly always quite small. . . . The germ cells of the pineapple are perfect and functional. When pollen grains are placed on the stigma of the same flower germination takes place and pollen tube growth starts but very quickly ceases, apparently due to some inhibiting influence of the style. This inhibiting influence, however, does not operate in cross pollination between varieties".

Aristolochia Sipho. Examining fruits of *A. Sipho* which apparently developed without fertilization, Maillefer (95) found that the ovules were reduced to a spongy mass without anatomical differentiation.

Artocarpus incisa (Breadfruit) is often seedless (6).

Capsicum (Pepper).   Hösterman (72) reports that late in the summer of 1912 he obtained parthenocarpic fruits in *C. annuum* without any special treatment.   The soil had been fertilized late and the plants were vigorous.   Next year he observed parthenocarpic fruits on two varieties.   Wölfert (161) obtained a few small, shrunken but seedless peppers by removing the vegetative buds and most of the flower buds.   Cochran (21) found that shifting *C. frutescens* plants from temperatures of 90–100° and 70–80° F. to one of 50–60° F. at the time of anthesis increased the percentage of fruit setting, and many of the fruits were parthenocarpic.   Anatomical examination showed that the development of parthenocarpic fruits was due not to lack of pollination but to slow growth of the pollen tubes which rarely reached the ovules.   The growth of pollen tubes in the style and upper part of the ovary was, however, enough of a stimulus to cause the latter to begin development into a fruit.

Carica (Papaya).   Normally papaya contains numerous seeds, but Heilborn (64) found two new species in Ecuador which were entirely parthenocarpic.   These species, which he named *Carica chrysopetala* and *C. pentagona,* were cultivated in the Andean valleys, and he neither saw nor heard of staminate flowers.   He found reduction division normal in *C. chrysopetala,* but in *C. pentagona* both normal and aberrant divisions took place.   He sometimes found ovules with two mother cells, one of which showed complete and the other incomplete conjugation.   The ovules with incomplete conjugation gave rise to diploid embryo sacs, which may perhaps be capable of apomictical development.

Higgins and Holt (66), working in Hawaii, made a number of crosses of *C. papaya* and obtained several trees grown from these crosses that produced parthenocarpic fruits when the pistillate flowers were covered with paraffined bags before anthesis.   In an orchard they also found a tree that produced seedless fruits whether pollinated or not, showing that this was an instance of vegetative parthenocarpy.   These authors conclude that parthenocarpy, though it occurs now and then, is not very common in *C. papaya.*   The parthenocarpic fruits had hollow centers, and as they make no mention of size it can be assumed that the size was normal.

Schaffner (128) made an interesting experiment with a pistillate plant growing in the greenhouse at Ohio State University.   The

flowers usually dropped as soon as they opened and there was never any growth of the ovary. He decided to remove all the larger leaves and he thus had a plant with a large root system but a very small transpiring area. He found that the flowers remained on the plant and the ovaries began to enlarge. The fruits were removed before being mature, but he states they were growing vigorously at the time of removal.

Casimiroa edulis (White Sapote). Lesley (90) informs the writer that at the Huntington Garden, San Marino, California, there is a tree of this species known as the Collins Seedless which bears small seedless fruits as well as larger seeded fruits. This tree was obtained from the Chico Experiment Station as U.S. PI 73081.

Citrullus vulgaris (Watermelon). According to Sereisky (131), Krovehenko found three seedless watermelons in a commercial field in 1932. The reviewer is not aware of any other report on seedless watermelons.

Citrus aurantifolia (Lime). The fruit known as the Tahiti lime has been reported by several investigators as entirely seedless (148a, 150). Uphof (150) made a cytological study of development of the pollen and the embryo sac. He found that for the most part microspore mother cells of normal appearance were produced, but further development did not proceed and in the mature anther the two locules had fused and only traces of dead protoplasm were observed instead of pollen. Development of the embryo sac did not proceed even as far as this. He was unable to find any differentiation of macrospore mother cells. There is, therefore, no doubt as to the reason why the fruit never produces seeds, even when cross pollination is employed.

Citrus grandis (Grapefruit, Pummelo). The Marsh grapefruit is more or less seedless, and Pope (119) states that in Hawaii it is usually seedless, but when present seeds vary in number from one to six. Reinking (122) reports that the double pummelo of Banda and Ambon is usually seedless and those fruits that are seeded have only a few seeds. Several investigators refer to the Siamese seedless pummelo (123, 47, 148a). In 1920 Reinking and Groff (123) visited Siam and found that at Nakorn Chaisri this variety produced seedless fruits, especially in June, but fruits ripening in November had seeds. These authors state that it is generally considered in Siam that the seedlessness is associated with saltiness of the irriga-

tion water.   From January to June when the seedless fruits develop, the irrigation water has an NaCl content of 1.95%.   It is also stated that if this variety is grown away from salt water the fruits become seeded and the quality deteriorates.   The flowers appearing in June at the beginning of the rainy season produce an abundance of fruit in November and these are seeded.   In a later report Groff (47), discussing the Kao Panne variety which he considers the best of the Siamese pummelos, states "seeds shrivelled, immature in the month of June, and scarcely noticeable".   This statement seems to indicate that the Siamese Seedless pummelo is not a parthenocarpic fruit but that fertilization takes place and the seeds abort at an early stage of development.   Torres (148a) writes that this variety of pummelo is seeded in the Philippines and that it is self-sterile.

Citrus hybrida.   Nagai and Tanikawa (107) found that when the varieties Yamabuki and Asahikan were selfed they produced an abundance of seedless fruits, whereas cross pollination resulted in seeded fruits.   Yamabuki produced fruits even when pollination was completely prevented.   No such experiments were reported for Asahikan.

Citrus Limonia (Lemon).   Several years ago the writer (52) saw a Eureka lemon tree in a private garden in Riverside, California, which was, by the owner, stated to produce only seedless fruits.   It was the only lemon tree in the garden, but no doubt others were near by so that probably cross pollination was effected. The flowers and fruits were entirely normal in appearance.   A study was made of the growth hormone content of the ovaries and it was found to be approximately twice as great as that from seeded Eureka lemon trees.   Nagai and Tanikawa (107) found that the Eureka, Genoa and Lisbon lemons set fruit without pollination, although the percentage setting was much lower than with pollination.

Citrus nobilis (Mandarin, Satsuma orange).   Nagai and Tanikawa (107) write that when the varieties Wase-Unshiu, Makaku-Kishiu and Kunembo were self-pollinated only seedless fruits were produced, and that most of the Unshiu fruits produced by self-pollination were also seedless.   The Unshiu and Kunembo produced seeded fruits when cross pollinated, but the Makaku-Kishiu produced seedless fruits even when cross pollinated.   Pope (119) finds

that in Hawaii the Satsuma (Unshiu) has normally either no seeds or only one to four.

Citrus sinensis (Common or Sweet orange). It is well known that the Navel orange is seedless but that other commercial varieties usually have seeds. Pope (119), reporting on Hawaiian oranges, mentions a number of varieties that have either no seeds or only a few: Navelencia, seeds none to few; Valencia, seeds none to few; Washington Navel, seeds none to few; Thompson Navel, seeds none to few; Buckeye Navel, normally seedless but may become pollinated from other citrus and produce one to 15 seeds. Nagai and Tanikawa (107) found that Washington Navel, Thompson Navel, Navelencia, Maltese Blood, Ruby Blood, Joppa, Valencia and Kwantung all produced fruits without pollination.

Webber was perhaps the first to study the cytology of the orange, and a quotation from his paper in the California Citrograph for 1930 (156) describes the condition in the Washington Navel, which is perhaps typical for other seedless varieties. Referring to his investigations in 1893 and 1894 he says: "A microscopic study of the anthers made at that time showed that the development of the pollen in the anthers proceeded apparently normally up to the pollen mother cell stage, but that then the development was arrested and the mother cells gradually disintegrated. It seemed to be impossible for the mother cells to initiate and go through the reducing division. These conclusions have since been entirely confirmed by Osawa and others and may be accepted as representing the normal conditions. The fact that no viable pollen is developed in the Navel has been confirmed by all investigators that have worked on the subject, among them, Ikeda, Osawa, and Frost. From this extreme degree of almost total sterility in the anthers of the Navel and Satsuma one can find in different varieties all degrees of sterility up to complete fertility. What has been said with reference to the sterility of the anthers applies equally well to the ovaries and egg apparatus. Varieties or strains of the Navel and Satsuma oranges develop very few perfect ovules and seeds, being thus practically seedless".

Concerning the development of fruits without pollination, again referring to his experiments of 1893 and 1894, Webber says: "I thus in my experimenting opened a number of Navel orange flowers last spring and summer (1893) before the pollen or the pistil had

matured, emasculated them (*i.e.*, cut off the stamens) and immediately drew over the flower thus treated firm paper bags or closely woven cloth bags and tied them around the branch below the flowers, so that all insects were excluded. A number of the flowers thus treated matured fruits which were to all appearances perfectly normal though developed, we can almost positively say, without the access of pollen. Again this spring (1894) a number of flowers were similarly treated and several have set fruits and to all appearances are developing normally though all pollen was excluded, and thus there was no fecundation. The Navel fruits developed last year from emasculated flowers on examination were found to be perfectly seedless, with the exception of a few small rudimentary seeds. Though my experiments have not been extensive enough to be conclusive it nevertheless seems from the results obtained that the Navel fruits possess the faculty of developing without the action of pollination".

Cucumis sativus (Cucumber). Noll (110) observed that the varieties Rytows Gurke and Grosse Schlangen produced seedless fruits when pollination was prevented. In some the ovules made a little growth but no embryos were observed. Many of his fruits grew to full size, while others were small. Munson (104) found that the varieties Blue Gown and Duke of Edinburg produced seedless fruits even when pollination was prevented. The fruits were frequently hollow at the flower end. The Sion House and a telegraph variety also produced 10% seedless fruits of normal size if the flowers were covered with bags before pollination took place. Tiedjen (145) found that cucumber vines grown in soil well supplied with nitrogen and under reduced light produced seedless fruits even when pollination took place. These same vines produced seeded fruits under optimum light conditions. It is known that pollen germination and tube growth are poor during cloudy weather and it is possible that only short pollen tubes developed under the low light intensity. While of insufficient length to bring about fertilization these pollen tubes may have been long enough to reach the upper part of the ovary and thus induce fruit development. Hawthorn and Wellington (62, 157) report the development of seedless varieties as a result of some breeding work they conducted. A good market fruit without seeds was developed as a result of crossing Arlington White Spine with Rockford Market. The former

crossed with an English forcing variety also produced a seedless form having good market qualities. Both of these parthenocarpic fruits were selected for several generations, and finally the parthenocarpic character was fixed so that seedless fruits were produced without pollination. McCollum (93) also reports parthenocarpic fruit development in certain strains of the White Spine type. Strong (137) found that of 34 varieties grown in the greenhouse all produced some parthenocarpic fruits when pollination was prevented. This is contrary to the finding of Wellington and Hawthorn (157). Only a few varieties, however, produced a high enough percentage of seedless fruits to be of commercial value. Suttons Delicacy with 75.7% of seedless fruits was one of these. In Europe Bremen-Estland (11) found several varieties of forcing cucumbers that produced seedless fruits without pollination. He states that in the western European technical (horticultural) literature it is always emphasized that the true forcing cucumber, which is of the long slender type, does not need to be pollinated to produce fruits. The writer has also grown the telegraph type in the greenhouse at Ann Arbor without pollination and the fruits were of normal size and seedless.

A report from John Innes Institute (3) states that all varieties of forcing cucumbers commonly used in England are parthenocarpic and set without pollination. It has been found that when grown at a high temperature they are seedless, even when pollination has been made, and in order to get seeds it is necessary to grow them at a low temperature and hand pollinate.

Cucurbita pepo (Pumpkin). Höstermann (72) reported that several varieties of pumpkin, when pollination was prevented by either cutting off the stigma or covering it with colored water glass before the flower opened, produced seedless fruits. Höstermann's experiments are of special interest in that he obtained no fruit development as a result of his treatment early in the growing season, but as the season advanced the ovaries developed more and more before they dropped off, and finally in October and November he harvested several fruits that from the weights he gives must have been of normal size or nearly so, but without any seeds. The varieties he used normally produced only a few seeds, *i.e.*, they were on the way toward a seedless condition.

Erwin and Haber (30) made a number of cross pollinations

between different varieties of *Cucurbita maxima, C. pepo* and *C. moschata.* Of 3000 such pollinations 369 fruits were obtained and 99% were seedless. Castetter (17) made similar crosses and records many fruits without viable seeds. This is of course not very enlightening so far as knowledge of embryo development is concerned, but the writer himself speaks of many parthenocarpic fruits being formed from these crosses.

Diospyros (Persimmon). In 1908 Woodburn (166) reported seedless fruits of *Diospyros virginiana* in Bloomington, Indiana. They were as large as the seeded ones, of good quality and as a rule ripened earlier. In 1911 in a second paper (167) he reports that morphological studies showed that no pollination had taken place, nor was there any embryo development, but considerable endosperm tissue developed at the time the eggs disintegrated. Miss Hague (59) also found seedless fruits at Decatur, Illinois. Microscopic examination showed no pollen tubes in the stylar tissue nor any fertilization. Yet when she covered branches so that insect visitations were prevented, no fruits were produced. She states that no staminate trees were nearer than two miles. Claypole (20) reports that he once saw a whole grove in Pennsylvania in which seeded fruits were the exceptions.

Diospyros Kaki, the Oriental persimmon, has been reported to be parthenocarpic by a number of investigators (46, 70, 75, 76, 142, 158). Wettstein (158) found a single tree producing only female flowers in the Vienna Botanical Garden and this tree produced seedless fruits that compared favorably as to size with fruits obtained from other regions. Hume (75, 76) states that when trees are far apart a whole tree may produce nothing but seedless fruits, and he is of the opinion that pollination does not increase fruitfulness in some varieties, but definitely so in other varieties. Tamari (142) investigated the varieties Dai-dai-maru, Emon and Hakogaki for ability to set fruit without pollination. Immature female flowers were bagged and produced fruits of normal size but without seeds.

In 1939 Hodgson (70) examined 167 trees from 63 accessions (about 50 different varieties) and found 15 trees bearing 100% seeded fruits, four trees bearing only parthenocarpic fruits and the remaining 148 trees with both seeded and seedless fruits. In a private communication he states: "Most of our commercial varieties —Hachiya, Hyakume, Tanenashi, Tamopan and Fuyu—are pistil-

late and hence usually have no opportunity whatever for pollination. They regularly and normally bear satisfactory crops of seedless fruits; indeed they often overbear.   So far as I can tell, without having bagged the flowers, all of the 50 or 60 varieties of *D. Kaki* in my collection bear seedless fruits when unpollinated and seedy fruits when pollinated".

Gould (46) states that in many varieties the flesh of the seedless fruits is light in color and astringent until the fruits are quite soft, while in the seeded fruits the flesh becomes dark and sweet even while the fruits are still hard.

Akh (1) reports that in South Chakeh in the Caucasus the Caucasian persimmon (*Diospyros Lotus*) is occasionally found to be seedless.   Thus he found either whole trees or certain branches with only parthenocarpic fruits, and these had produced only seedless fruits for many years.   Hodgson writes: "From my work with persimmons I believe it safe to say that *Diospyros Kaki* and *D. Lotus* regularly exhibit parthenocarpy in California, and presumably so elsewhere".

Dodonaea viscosa.   This evergreen shrub of India is reported by Joshi (81) to produce parthenocarpic fruits, without pollination. The fruits are of normal size but the style remains green and persists for a longer time than in seeded fruits and may even grow in length.

Eugenia.   Pijl (117) reports from Bandoeng, Dutch East Indies, that *Eugenia aquea* produces an abundance of seedless fruits, and as these trees produced no pollen we have an instance of vegetative parthenocarpy.   Examinations of the ovaries showed that at least some of the embryo sacs were normal.   In *E. malaccensis* he found a few seedless fruits, apparently as a result of self-pollination. The same author found that *E. javanica* pollinated with *E. jambos* produced a few seedless fruits.   *E. javanica* also produced seedless fruits when the style was cut to prevent pollination.   This plant thus also is able to produce fruits without the stimulus of pollen.

Euphorbia dulcis var. *purpurata* produces fruits parthenocarpically, even though it may have seeded fruits (16).   According to Carano, the embryos develop adventitiously from the nucellus; most of the microspore tetrads degenerate and the few pollen grains produced are sterile; megasporogenesis is also irregular.   This is considered to be a case of parthenocarpy, even though the fruits may

have seeds, because the fruits were produced without pollination and fertilization.

Ficus carica (Fig). Condit (25) recognizes four general horticultural types of figs: the Caprifig, Smyrna, White San Pedro and the Common. Of these the Caprifig is the only one that bears mature stamens, and the Smyrna and the second crop of the White San Pedro are the only ones that require pollination and fertilization for fruit formation, *i.e.*, they are not parthenocarpic. The Caprifig, which harbors the fig wasp necessary for pollination of the Smyrna type, produces two or three crops each season. The first crop, the "profichi", are of two types, the "insectiferous" which contain the fig wasp, and the "polleniferous" which do not have the wasp but an abundance of pollen. As the latter fruits have no wasps they are of no value in a commercial orchard. The female wasp from the "profichi", carrying pollen on its body, enters the syconium of the Smyrna type and brings about pollination which results in fruit formation. The Caprifig produces two more crops of fruit, the "mammoni" and the "mamme", both of which require the presence of the fig wasp larvae and pupae for fruit formation. All three crops of Caprifigs, with the exception of the "polleniferous", then require stimulation of the fig wasp for fruit formation and are examples of stimulative parthenocarpy. There are, however, Caprifigs which produce fruits without stimulation, such as the Cordelia which is identical with the Croisic of Europe. The Cordelia and the "polleniferous" "profichi" are illustrations of vegetative parthenocarpy.

The first crop of White San Pedro is parthenocarpic but the second crop requires pollination and fertilization, and the fig wasp is here also responsible for pollination. The Common type produces fruits without pollination. The Smyrna, as exemplified by the variety Calimyrna, may sometimes produce a few fruits in the early crop without pollination. We thus have in the fig both vegetative and stimulative parthenocarpy. According to Condit, the endosperm may develop to quite an extent in some of the parthenocarpic fruits.

Fuchsia sp. Wölfert (161) was able to cause parthenocarpic fruits of normal size to develop in the variety Neue Welt by removing all but one bud from a flower cluster.

Garcinia Mangostana (Mangosteen). In a recent note Horn

(71) states that his examination of two 37-year old trees in Puerto Rico shows that no pollen is produced, yet about half the fruits have one or more seeds. No staminate trees have been found in Puerto Rico so there can be no question of pollen being carried from a distant tree. Sprecher, according to Horn, found that in the mangosteen "the seed originates from a cell in the epithelium of the ovary inner integument. The reproduction is thus distinguished from nucellar budding that occurs in the apogamic reproduction in the Mango and Citrus". Even though seeds are sometimes found in these fruits, they must, nevertheless, be considered as having arisen parthenocarpically, as there was neither pollination nor fertilization.

It would be interesting to speculate about the number of species of plants with seeds that may have parthenocarpic fruits. Only cytological studies of developing fruits could give this information. As mentioned above, the mango and certain varieties of orange are known to produce seeds from cells other than the fertilized egg, but how many other species there are we do not know.

Hedyosmum brasiliense. In 1890 Müller wrote Kronfeld (85) that he had in his garden two young trees of *Hedyosmum brasiliense*. Both were pistillate and no staminate trees were nearer than 30 kilometers, yet these trees produced fruits with seeds that looked normal until they were cut, when it was seen that they were empty.

Hesperis tristis. In his private garden at Freiburg, Hildebrand (69) had a single specimen of this plant, which blossomed profusely but set only a few fruits, and these were seedless. This plant is known to be self-sterile.

Impatiens. In his studies on parthenocarpy Wölfert (161) found that he could cause seedless fruits to be produced on *Impatiens Oliveri, I. Holstii, I. Sultani* and a Sultani hybrid by removing most of the flowers and cutting off the apex of the plant. The flowers were covered to prevent pollination. This experiment was repeated a second year. He also produced parthenocarpic fruits by cross pollinating these species with one another. The seedless fruits produced were somewhat smaller than normal fruits.

Lobularia maritima (= *Alyssum maritimum*). In his Freiburg garden Hildebrand (69) had left from many specimens only a single plant which blossomed profusely but produced only a few fruits. All these fruits were entirely seedless.

Lycopersicum esculentum (Tomato). As will be seen from the following notes, tomatoes which are ordinarily quite seedy may under certain conditions produce parthenocarpic fruits. Thus Sandsten (125) found that when he grew Earleana plants in excessively rich soil in the greenhouse he obtained a number of abnormal plants, and of these two produced parthenocarpic fruits. The fruits from one plant were of normal size, but the other plant produced small fruits. The plant that produced the large fruits had only a few blossoms with abnormally large and thickened pistils, and the stamens produced no or very little pollen. The other plant had normal flowers. The large seedless fruits were composed of solid flesh throughout without any differentiation into pericarp and seed cavities. The small fruits were sometimes solid and other times they had small locules. Cuttings from these plants set out in the field during summer continued to produce seedless fruits.

In his studies on the injurious effects of premature pollination in tomatoes, Hartley (60) found that a few seedless fruits developed as a result of rubbing the stigma in powdered $MgSO_4$. During the past year the writer has repeated Hartley's experiment with $MgSO_4$, using the John Baer tomato, without ever having noted the slightest growth of the ovary as a result of the treatment; but then, during a period of over 15 years no naturally occurring normal-sized seedless fruit has been observed in this variety, although thousands of fruits have been sliced open for observations, and, as Hartley noted seedless fruits produced without pollination or any other treatment, in his plants, it is very likely that the variety Lorillard naturally produces a few parthenocarpic fruits, and that the fruits Hartley thought were produced as a result of his $MgSO_4$ treatment would have been produced without this treatment. During the past summer (1941) the writer found a few seedless fruits of the variety Valliant. These fruits were only slightly smaller than the seeded fruits and were not distinguishable from them until sectioned. They were produced without any treatment of the plants or flowers.

Höstermann (72) experimented with a number of varieties of tomatoes for ability to produce seedless fruits. His procedure was to emasculate the flower buds, remove the corolla and cut the style, thus effectively preventing pollination. These treatments were carried out at four different times during the summer. Most of his plants produced no fruits as a result of the early treatments, but those

made later in the season gave some results.   While his results were not promising from the standpoint of obtaining a variety that would produce seedless fruits, they are, however, of considerable interest. From Earleana he obtained 43 seedless fruits with well developed placentae and seed cavities filled with mucilage.   Earliest Red also produced seedless fruits.   The smaller were of solid flesh, while the larger had the locules well filled with mucilage, but even in these the percentage of flesh was greater than in the seeded fruits.   In this variety he obtained two seedless fruits even when pollination had not been prevented.   Five Ficarazzi plants out of 16 treated produced 24 parthenocarpic fruits that had underdeveloped locules with very little mucilage.   Six plants of Johannisfeuer produced 25 seedless fruits, and as all but two were picked green no information about amount of flesh was obtained.   Königin der Frühen, Alice Roosevelt, President Garfield, Ponderosa, Mikado and King Humbert all produced a few small parthenocarpic fruits when pollination was prevented.   Comet, Lucullus and Sterling Castle produced some parthenocarpic fruits even when pollination was not prevented.

That environmental conditions are important in natural production of parthenocarpic fruits in tomato has been shown by several investigators.   Hawthorn (61) reports that a cross between Large Cherry and Bonny Best resulted in selections which produced seeded fruits in June and early July, seedless fruits during the hot part of the summer and again seeded fruits in the fall.   During the period of production of seedless fruits the plants continued to bear as profusely as at other times and the fruits were of good quality. These studies were made in the Winter Garden Region of Texas. In their study of tomato "pockets", Foster and Tatman (38) found that high or low temperature, low carbohydrate and nitrogen reserves were causes for production of parthenocarpic fruits.   Low carbohydrate reserve was caused by high soil nitrogen, high soil moisture, high temperature and short days.   Lack of seed production under high or low temperatures, as found by these investigators, is no doubt explained by the experiments of Smith and Cochran (133a) who found that at 50° and 100° F. pollen germination and tube growth is very low, whereas at 70°–85° F. germination is quite good and tube growth greater than at the other temperatures. Under the unfavorable conditions mentioned above, some pollen germinates and produces pollen tubes which may never reach the

ovules, but Yasuda (171, 172) has shown that pollen tubes in the style or ovary are sufficient in many plants to start the ovary growing into a fruit, even though no fertilization has taken place.

As shown by Lesley and Lesley (91), plants producing parthenocarpic fruits may have a deficiency in their chromosomes. These investigators state: "A very small tomato plant originated in an F_1 culture from diploid parents. It had an unequal pair of homologous chromosomes and proved to be a deficiency heterozygote, about one third of the chromosome being deficient". They also had originated "a primary trisomic, a deficient trisomic having an extra chromosome similar in size to the deficient chromosome, and a deficient tetrasomic from selfing the deficient plant; and a secondary having a ring trivalent originated from crossing it with a diploid. The deficient plant and all its aneuploid derivatives were very fruitful. Fruitfulness was associated with a tendency to parthenocarpy. Without artificial pollination most of the fruits of the deficient plant and of the deficient tetrasomic were parthenocarpic. But with artificial pollination they were as a rule seedy—even when self-pollinated. On deficient trisomic and primary trisomic plants parthenocarpy was frequent in the basal fruits but gradually less so in fruits that set on later-developed inflorescences. Parthenocarpy was stimulative except in the deficient tetrasomic which set fruit without pollination". These plants, while fruitful, grew very poorly and the fruits were small.

Many times the writer has observed, in the variety John Baer, very small fruits produced in clusters that had long since matured their fruits. These fruits, never as large as two centimeters in diameter, are composed of solid meat without any trace of seeds. They originate from flowers that probably are not pollinated or at least not fertilized, and that for some reason do not absciss but remain on the plant until all other fruits have matured when a rather high nutrient level is produced in the plant as a whole. In a private communication Roberts mentions having seen similar fruits, and Dr. Janes, a student in our department, has found some too.

Many years ago when graft hybrids were especially intriguing, Winkler (160) produced a number of such hybrids between *Solanum nigrum* and different varieties of tomatoes. Many of these produced parthenocarpic fruits, some with and others without pollination.

Mamillaria Wildii.   When he prevented self pollination Wölfert (161) obtained normal-sized seedless fruits in this plant.   Cross pollination also resulted in parthenocarpic fruits.

Mespilus germanica (Medlar).   Wölfert (161) reports that in the Botanic Garden at Kiel there are seedless fruit-bearing trees next to trees that bear seeded fruits.   Flowers of the seedless variety lack stamens.   Bailey (6) also mentions a seedless variety of Medlar.

Morus nigra (Mulberry).   According to Kronfeld (85), Camerarius reported that an old tree of *Morus nigra* after a pause of many years again began to bear an abundance of fruits, but they were all seedless.   Claypole (20) also reports a mulberry tree which, though a long distance from staminate trees, bore an abundance of fruits, and they were all seedless.

Musa sapientum (Banana).   If there ever was a seedless fruit it is the banana of commerce.   In the common banana there are three sets of flowers produced in each cluster.   The basal flowers which are the first to open are pistillate and grow into the fruit; the central flowers are usually perfect but do not produce useable fruits; the terminal flowers which do not all open before the fruits are mature are predominantly staminate (7). D'Angermond (2) determined by two different experiments that pollination is not necessary for fruit production in the banana. In the first experiment he covered the flowering stem with a bag before the flowers opened; all staminate flowers had first been removed.   In the second experiment he covered the flowering stem as before and also removed the perianth, staminodia and the style before the flowers opened.   In both experiments normal fruits were produced.   Very few pollen grains germinated in a hanging drop and he found that there were hardly any ovules with embryo sacs, even when the nucellus and integuments were normal.   Cytological studies further showed that sometimes the embryo sac mother cell divided normally and at other times abnormally. Tischler (146) found that with increase in the number of chromosomes there appears a disturbance in pollen development.   The chromosomes do not separate properly in the hetero- and homotypic divisions; some lag behind and form separate nuclei, and a single mother cell may form eight pollen grains.   That some normal ovules are formed is proven by the fact that when the common banana was

pollinated by *M. Basjoo* or *M. rosacea,* fruits with a few seeds were formed, but the reverse produced no seeds.

D'Angermond also found that *M. Cavendishii,* the dwarf Jamaica banana, behaved similar to *M. sapientum,* but that *M. rosacea* and *M. Basjoo* required pollination for fruit setting and these fruits contained seeds.

Nicotiana tabacum (Tobacco).   Hartley (60) found that when the pistil of a Cuban variety of tobacco was treated with corn flour, air-slacked lime, $MgSO_4$ or *Azalea* pollen instead of tobacco pollen, a few small seedless fruits were formed, but as similar fruits were formed with the flowers emasculated and bagged, this is undoubtedly an instance of vegetative parthenocarpy.   Tollenaar and Middleburg (148), reporting some breeding experiments in the Dutch East Indies, state that while parthenogenesis occurs rarely if ever, parthenocarpy is fairly common.   By preventing pollination Savelli (127) obtained parthenocarpic fruits of all sizes in the varieties Sumatra Deli and Fumo de San Paulo.   Goodspeed (45) found that from 800 castrations and mutilations 100 fruits matured.   In the majority of these parthenocarpic fruits, empty seeds were produced in great numbers.   Some were as large as normal seeds, though the majority were smaller.   Few flattened and shrivelled seeds were formed.   From nine capsules he obtained 50 seeds which he thought had normal embryos and endosperms.

Olea europea (Olive).   Campbell (15) reports a very interesting case of parthenocarpy in the olive.   On June 6, he states, the orchard was enveloped in a heavy fog.   A few days later only a few flowers showed any sign of ovary growth; all the others were completely closed, which was a sign that no fertilization had taken place.   Most of the latter dropped off very soon.   The flowers that remained on the trees grew, and it soon became apparent that fruits of several sizes were developing.   During the growing season he made occasional observations on these fruits.   During the dry weather of early September, many of the smaller fruits dropped off, but as soon as the rains came this ceased.   Examining the mature fruits, he found that the large fruits had seeds.   The smaller fruits, which ranged in size from one-third to one-half the size of normal fruits, were without seeds.   The larger of the seedless fruits had a well developed hard endocarp and a poorly developed mesocarp. In the smallest fruits the mesocarp developed well, but the endocarp

poorly.   The seedless fruits were round, whereas the seeded fruits were oval in shape.   How the fog could have produced the parthenocarpic fruits Campbell does not explain.   However, he states that on examining the flowers after the fog he found that the anthers were dry and there was no sign of pollination.   Campbell's observations were made in Italy.

Pirotta and di Pergola (118) also noted large and small fruits in the olive; the small fruits were seedless.   Cytological studies showed that development of the female gametophyte is quite irregular.   During development of the macrospore, the nucellus is digested and the macrospore passes into the micropylar canal and there disintegrates.   They found no traces of pollen tubes.

Oryza sativa (Rice).   Nagai (106) found a mutant of rice that was completely sterile.   The sterility was due to lack of fertile pollen.   He observed parthenocarpic caryopsis in these plants.   Morinaga and Fukushima (99) observed the same phenomenon in some haploid plants.

Papaver Rhoeas (Corn Poppy).   Wölfert (161) produced parthenocarpic fruits by enclosing castrated flower buds in parchment bags.   The fruits were about one-third the size of the seeded capsules.

Persea gratissima (Avocado).   As obtained on the market the avocado has a large seed, but occasionally there are seedless fruits.   Instead of being pear-shaped these resemble more a cucumber and hence the name "cucs".   The writer has bought these "cucs" on the market in Pasadena, California, and found them very delicious, even more so than the seeded fruits.   In a private communciation Professor Hodgson from the Division of Subtropical Horticulture, University of California at Los Angeles, California, has the following to say concerning the prevalence and formation of seedless avocado:

"Seedless fruits are quite common in the Fuerte and Matney varieties and are occasionally found in others.   I have cut hundreds of seedless fruits and have never found the seed cavity entirely empty, from which I conclude that embryo development starts but stops short of seed formation.   I believe that embryo-sac disintegration occurs or abortion after fecundation and that this takes place after pollination but before fecundation or after the latter.   From my work on the bearing behavior of the Fuerte variety I have

reached the conclusion that mean temperature during the pre-flowering, flowering and fruit-setting period is the primary factor responsible for the production of seedless fruits. I think it inter-feres with the development of the embryo-sac, delays the growth of the pollen tube, and influences the growth of the embryo.

"In this connection I think it should be noted that this phenomenon is confined almost entirely to varieties that blossom in mid-season (winter) when temperature conditions are most unfavorable. It is much more prevalent in seasons of below-average mean tempera-tures. Moreover, certain localities regularly exhibit much more of it than others and the records indicate that these are areas of below-mean temperature for the avocado-growing sections as a whole".

Phillyrea media. Like its cultivated relative, the olive, this plant also produced fruits of two sizes. The large ones are seeded and the small ones seedless. Campbell (14) points out that, as in this wild species there is also parthenocarpy, the parthenocarpic condition in the olive cannot be due to cultivation.

Phoenix dactylifera (Date Palm). In his paper on "Direct effect of pollen on the fruit of the date palm", Nixon (109) states that the sugar content of three lots of parthenocarpic dates was essen-tially the same as that of seeded fruits. In the same paper he shows photographs of seeded and seedless fruits. The latter are slightly smaller but there is no difference in appearance. A quota-tion from a private communcation from Nixon gives further informa-tion on parthenocarpy in dates:

"Seedless fruits are of fairly common occurrence in *Phoenix dactylifera.* Usually such fruits do not develop unless the set is very poor on a given bunch. There may be a slight enlargement of all three carpels of the flower, or of only one. Whether all three or only one of the carpels develop is partly a varietal character, but other factors are apparently involved as both types of fruits may occur on the same cluster. Seedless fruits are slower in developing and usually smaller than normal, seedy fruits; they may eventually ripen but are almost invariably coarse in texture and lacking in flavor. I have occasionally seen seedless fruits that were edible but none that could compare with the seedy fruit of the better im-ported varieties. From time to time in localities where dates are not grown commercially seedling date palms are found bearing edible seedless fruits and sometimes this results in newspaper write-ups about a seedless date variety.

"The fact that seedless fruits do develop from flowers from which pollen has been carefully excluded indicates that seedless date fruits can be parthenocarpic. Whether the seedless fruits that develop on bunches with a small percentage of pollinated flowers setting fruit are the result of some pollen stimulus or are vegetative parthenocarpic is not known".

Pisum sativum (Peas). Haan (57) found, in what he called the slender pea, that emasculated flowers produced seedless pods to the extent of about 30%. He had previously observed that out of 999 pods collected from his plants 310 or 38% were entirely empty. From his emasculation experiments it appears that these were naturally parthenocarpic. The phenomenon of natural parthenocarpy is then quite common in this variety of pea, and it would not be a profitable variety to grow.

Prunus persica (Peach). Stewart and Eustace (135) reported in 1901 the production of small peaches in the variety Globe and to a slighter extent in the Elberta. These fruits colored and remained on the trees till September. Some had hard pits and others only soft ones that could be cut with a knife. No seeds were, however, found. Several fruit growers reported to them the finding of similar small peaches. Shing and Feng (132) noted small and large fruits on the same branches in the variety Shen-Chou, and Dorsey (28a) finds that in some years these small fruits or "buttons" are very common in the Hale peach. Shing and Feng selected a number of blossoms in spring and treated them as follows: emasculated and bagged, cross pollinated with Lu-Yuehhung pollen, or left for normal pollination. Thirty-four per cent of the flowers emasculated and bagged produced parthenocarpic fruits, 85% of the flowers pollinated with foreign pollen produced parthenocarpic fruits, and normal pollination resulted in the setting of 34% to 42% parthenocarpic and 8.6% to 12% normal fruits. These authors found that growth of the small parthenocarpic fruits was the same as that of the large fruits except that it was slower throughout the season and their ripening was later. Dorsey made cytological studies of developing fruits from the blossom stage to mature fruits. From these studies he draws the conclusion that the "buttons" are formed as a result of fertilization and abortion of the embryo at a very early stage of development. The evidence which he is able to present for fertilization is, however, indirect.

Prunus cerasifera (Cherry Plum). From the John Innes Institute (3) comes the report that when the variety Red 2X *Prunus cerasifera* is pollinated by *P. domestica 6X,* seedless fruits are produced, which ripen earlier than the seeded fruits, when this variety is selfed.

Pyrus communis (Pear). Parthenocarpy seems to be quite common and widespread in this plant, as indicated by the following references. Sturtevant (140) writes that such delicate varieties of pears as the Gansel's Bergamot and the Chaumontelle have rarely any seeds. Belle de Bruxelles is always seedless. Waite (154), in his studies on apples and pears, selfed many varieties, and when selfed pears produced fruits they were more slender than those produced by crossing and as a rule were seedless. He thinks that there was no fertilization but that pollination stimulated the ovary to growth.

Ewert (32), who was very much interested in the development or discovery of varieties of seedless fruits, had a very clever way of preventing pollination without emasculating and enclosing the flowers in bags or nets, which is very time-consuming. He placed a solution which he called "Kernloss" upon the style. This solution prevented pollination, both physically and chemically. As frequent references are made to it in the literature of the time its composition will be given. "Kernloss" was made by dissolving 50% sodium silicate, 1% Nigrosin, 1% eosin, and 1% $CuSO_4$ in a weak ammoniacal solution. This solution killed the style and coated it with a layer of sodium silicate; the $CuSO_4$ prevented infection by fungi. From our recent knowledge of stimulation of parthenocarpy by chemicals, one wonders whether this chemical might not have done that too. From his result with emasculation experiments, in which pollination was prevented by covering the blossoms, it does not seem to the writer that there was any such stimulation. He found that the two varieties Clairgeau and Gute Louise von Avranches produced an abundance of seedless fruits when pollination was prevented by either emasculation or treatment with "Kerloss". Other varieties, like Holzfarbig Butterbirne, Nina, König Karl von Wurtemberg and Abbé Fétal, also produced a few parthenocarpic fruits. As a rule seeded fruits were produced in these varieties with cross pollination. Müller-Thurgau (101, 102), working in Switzerland at about the same time that Ewert was working in Germany and us-

ing the latter's methods, had two varieties of pears that produced seedless fruits when pollination was prevented. These were the Lebrun Butterbirne and the Rostiezer.

In the spring of 1913 Höstermann (72) observed the destruction by frost of the stamens and the stigma, but not of the ovary, in Lebrun's Butterbirne. Many of these flowers produced fruits that were without core or had only slight development of the core, and all were seedless. These fruits were somewhat less round than the seeded fruits and the author emphasizes that they were larger than the seeded fruits. This is very unusual. Kraus (84), in a paper on self-sterility, pictures a fruit of Winter Nellis without any seeds and hardly any core, which was produced by self pollination. The fruit is of normal size and appearance. Wölfert (161) received a seedless pear from a tree in Wedel that bore only seedless fruits. The core was entirely replaced by flesh, but he states the taste was not good. The writer has received an advertisement concerning a seedless pear from a nursery in southern Michigan. The fruit is pictured as entirely seedless and coreless with a delicious flavor. It has, however, been impossible to obtain any fruit for examination.

Reinecke (121), working in South Africa, was interested in the effect of selfing upon embryo development in pears. He used a number of varieties and determined the percentages of seedless fruits produced, which are: Bon Chretien, 96%; Beurre Boge, 94%; Clapp's Favorite, 100%; December, 100%; Triomphe de Vienne, 100%; Beurre Hardy, 92% and Beurre Diel, 100%. He does not state the percentage of setting but from his discussion it would seem to be quite high. To determine whether these fruits were vegetative or stimulative parthenocarpic, he emasculated 115 flowers of the Kieffer and 48 of Clapp's Favorite, and left them unpollinated. Only two Kieffer and four Clapp's Favorite fruits were produced. He comes to the conclusion that with pears parthenocarpy is the rule rather than the exception in South Africa. This refers perhaps to such varieties as Kieffer and Bon Chretien, rather than to all varieties. He noted that the seeded pears had greater development near the carpels and less at the stem end than the seedless fruits. This has been noted by other investigators too. The storage life of the seedless fruits was less than that of the seeded fruits, and their quality, as shown by chemical analysis, was not so good as that of seeded fruits. This is contrary to the findings of Ewert and Müller-Thurgau who found that seedless fruits contained more sugar.

Bugini (12) reports that at the Stazione Sperimentale di Ortofrutti-cultura di Milano he encountered parthenocarpy in the varieties Abbé Fétal, Alexander Lucas, Clairgeau, Butirra Diel and Bergamotte Esperens. In his experiments he both castrated the flowers and covered the branches with large boxes covered with gauze to prevent insect pollination. He used Ewert's "Kernloss" but did not think it suitable for his experiments. Bugini considers that the nutrition of the tree is a very important factor in the production of parthenocarpic fruits in pears and apples.

A recent note from the John Innes Institute (3) states that Doyenné du Comice has produced only seedless fruits when pollinated with pollen from Beurre Bedford. Pollen development is abnormal in this variety and giant tetraploid pollen is formed. In culture these germinate to form branched pollen tubes. This is very likely an instance in which pollen tube formation takes place but no fertilization due to abnormalities of the pollen.

Pyrus malus (Apple). Sturtevant (140), in his discussion of seedless fruits, mentioned various writers who had seen or heard of seedless apples and he himself had seen such fruits in Massachusetts. Some of these had a core and others were devoid of a core; all were seedless. He also reports that Knight obtained seedless fruits when he grafted apple upon pear. Waite (155) obtained seedless fruits of about two-thirds normal size when he selfed Baldwin Apples. Ewert (31), following the procedure which has already been outlined under pears, obtained parthenocarpic fruits in Cellini, Charlamowski and Wintergoldparmäne. The first mentioned produced the greatest percentage. The parthenocarpic fruits of Cellini were of approximately the same weight as the seeded fruits produced by cross pollination. They were a little longer and had a core which was poorly developed but never entirely absent. Ewert made a very interesting observation concerning production of parthenocarpic fruits, namely, that if flowers on some branches of a tree were prevented from being pollinated while others were pollinated, the former branches produced no fruits, but an abundance of seeded fruits were formed from those blossoms that were pollinated. If only unpollinated flowers were left on a tree, however, seedless fruits were formed. Apparently parthenocarpic fruits were produced in these varieties only, when not in competition with seeded fruits. This will be discussed in greater detail in a

later section.   He found that the parthenocarpic fruits ripened
earlier than seeded ones of the same variety.   Müller-Thurgau
(102) had the variety Sonderkern produce seedless fruits, and as
there were no ovules in the ovaries there evidently was no fertiliza-
tion.   He did not know whether pollination had been accomplished.
Reinecke (120) found that the varieties Wemmershoek, Koo and
Versfeld produced seedless fruits when flowers were enclosed in
bags.

Bugini (12) has obtained parthenocarpic fruits in the Collins,
Renette del Canada, White Culvilla, Renetta Orleans, Flabevy and
Angelo Longone.   He usually enclosed branches in large boxes cov-
ered with gauze to prevent insect pollination.

Varrelman (152) reports that the Navels (var. *apetala*) : Wel-
lington, Spencer and Navel No. 3, usually produce an abundance of
parthenocarpic fruits under normal conditions.   Longo (92) has
made similar observations on other Navels.   Yet when the flowers
were bagged no fruits were developed except in some bags of Navel
No. 3 that became infested with aphids, where there was 100%
setting.   Another year the Spencer and Navel No. 3 were deliberately
infested with aphids and again the Navel No. 3 produced partheno-
carpic fruits but the Spencer produced no fruits.   The Navels are
pistillate and, according to Longo, blossom later than other apple
varieties and are not visited by insects.   Therefore there should
be no pollination under normal conditions.   It is for that reason
difficult to understand why they should not have produced fruits
when the flowers were enclosed in bags.   It is possible that the
bagging itself was detrimental to setting.

In some of his observations Heinicke (65) found that apple blos-
soms infested with aphids developed fruits, even when pollination
was not accomplished.   These fruits were very irregular and seed-
less, but they remained on the tree until mature.   The aphid in-
festation frequently began quite a while before the buds opened.   It
is not of course very surprising that aphid stings should stimulate
an ovary to develop into a fruit, since Link (91a) has shown that
aphids contain a high concentration of growth hormones.

Ribes sp. (Gooseberries).   Ewert (33) cut off the flower buds
of gooseberries just below the anthers and smeared the cut surface
with his "Kernloss", after which the plants were put under wire
netting to further guard against pollination.   The branches were

ringed below the flower clusters. Controls were used. Seedless fruits were obtained in abundance on the experimental plants and seeded fruits on the controls. One variety, the Red Triumph, produced seedless fruits about three-fourths as large as the seeded ones, and the other variety produced fruits of normal size. The parthenocarpic fruits matured about 14 days earlier than the seeded fruits, and were richer in sugar and more acid than the latter. The interior of the seedless fruits was completely filled with cells which were larger than those in the interior of the seeded fruits.

Rosa (Rose). When stamens were removed from buds and the flowers placed in parchment bags, Gustafsson (48) found that *Rosa canina* formed an abundance of parthenocarpic fruits, but only 6% of flowers so treated of *R. rubiginosa* formed seedless fruits.

Solanum melongena (Egg Plant). Bailey and Munson (4) obtained perfect but seedless fruits when flowers were castrated and covered with paper bags to prevent pollination. Next year Munson (103) repeated these experiments with similar results. Munson used hybrids and suggested that the vigor may have been responsible for the parthenocarpic development. Höstermann (72) also obtained parthenocarpic fruits in several varieties of egg plant.

Solanum muricatum (Pepino, Melon Pear). Munson (104) states that this plant blossoms freely and under proper conditions sets a considerable amount of fruits which, however, are invariably seedless. Bailey (6) lists this as a seedless fruit.

Solanum nigrum. By removing all but one flower bud and all shoots from a plant and castrating this one flower, Wölfert (161) was able to produce a seedless fruit of half normal size. It ripened a little late. Repetition of this experiment a second year produced similar fruits. If more than one flower was left on a plant they all dropped.

Spondias Mombina (Spanish Plum). Juliano (83) found that this plant produced parthenocarpic fruits as a result of lack of pollination. No pollen was produced because of degeneration of the microspore mother cells. A normal female gametophyte was produced. Though the fruits were seedless, a mature stone was produced.

Stratiotes aloides. Bequinot (9) reported that of an introduction of this plant to the lakes of Mantua only the pistillate plants remained, and they produced parthenocarpic fruits.

Tamarix dioica. Joshi and Kajale (82) made the following observations on development of fruits of *Tamarix dioica* in the absence of pollination. "The embryo sacs degenerate after some time and no embryos are formed, but the ovules develop some characters of the seeds. The epidermal cells at the chalazal end of the ovule elongate, divide, and develop into a tuft of long multicellular uniseriate hairs which are characteristic features of the seeds in the genus. The ovary also begins to increase in size and develops into the fruit, which ultimately dehisces by three valves like the normal fruits dispersing the plumed seeds, although these are without embryos. Starch is deposited in the embryo sac and cells of the nucellus, testa and pericarp as the embryo sac degenerates. This is probably correlated with the non-development of the embryo".

Theophrasta crassipes. Wölfert (161) observed seedless fruits in this species during two successive years but made no investigation as to their formation.

Vitis spp. (Grape). Seedless grapes are common enough on the fruit market, but it is surprising to learn the number of varieties that produce seedless fruits. Bioletti (10) mentions: Sultanina (synonymous with Thompson, Thompson's seedless, Lady de Coverly, Sultanieh, Oval-fruited Kishmish); Rose Sultanina; Giant Sultanina; Sultana (synonymous with seedless Sultana, Round-berried Kishmish); Black Corinth (synonymous with Zante Currant, Panariti, Passerina nera); White Corinth (synonymous with Passerina bianca) and Seedless Muscat. The last mentioned is, however, usually seeded. According to Olmo (113), there are two types of seedless grapes. The one represented by Black Corinth, Hunisa and Parthenocarpic Sultanina is parthenocarpic, while that represented by Sultanina and Black Monukka is produced as a result of fertilization and early abortion of the seeds and is not parthenocarpic but stenospermocarpic. As a rule the seedless fruits are smaller than the corresponding seeded fruits.

Reports of parthenocarpic grapes are scattered through the literature and only a few will be mentioned. Maheshwari (94) reports that Millardet induced the formation of seedless fruits in certain varieties of European grapes by pollination with *Ampelopsis hederacea* pollen. Susa (141) was able to produce parthenocarpic fruits in White Corinth, Black Monukka and Sultanina by emasculating and bagging the flower clusters.

According to Stout (138), "Negrul reports that the grape Taokvery, which has the imperfect hermaphrodite type of flowers, develops berries with empty seeds when there is no pollination, and that when there is self-pollination the berries may have empty seeds, or seeds with an endosperm, or seeds with an embryo. Negrul believes that the pollen of the imperfect hermaphrodite flower cannot function in fertilization but can exert a stimulative influence; hence he lists the variety Taokvery as exhibiting autonomic parthenocarpy". Negrul further found that the Muscat of Alexandria, after emasculation and without pollination, produced some berries without seeds, some berries with empty seeds, some berries with seeds which had an endosperm and some berries that contained an embryo. Self-pollination gave the same range of berries. According to Negrul, there is in this grape facultative parthenocarpy, of both the vegetative and the stimulative types. Olmo (113) reports an entirely parthenocarpic bud mutation from Sultanina. The berries are round and somewhat smaller than the parent type. The pollen is perfectly normal in appearance but germination is very low. He thinks the ovules must be abnormal because even when the flowers intertwine with those of the parent vines the resulting berries are parthenocarpic.

Several investigators have found that ringing the cane below the flower cluster is favorable for development of parthenocarpic grapes. Müller-Thurgau (101, 102) found that when stems of the Räuschling grape were girdled the number of parthenocarpic fruits increased very materially, and he associates this with increase in sugar content of the stem above the girdle. Paulsen and Berna (114) also found an increase in parthenocarpic fruits in the Climinita on girdling the stem. Jacob (78), Olmo (113) and Pearson (116) noted similar results with other varieties.

The cytology of development of the ovules and seeds of grape has been studied by Pearson (115, 116) in several varieties. The White Corinth, she finds, has a misshapen ovule with only the outer integument and an overgrown nucellus, but no embryo sac. Pollen tubes were found in the base of the locule, but as there is no embryo sac there can be no fertilization and the fruits are produced parthenocarpically. The Black Corinth also lacks an embryo sac, and from a study of a large number of berries she found only 16 seeds that germinated. In other instances the ovule had enlarged

considerably, forming a structure that looked like a seed but which on sectioning showed complete absence of an embryo. Pearson therefore considers that this grape is also parthenocarpic. The varieties Sultanina, Sultanina Rose, Sultanina Gigas, Sultana and Monukka have as a whole quite abnormal ovules, and many abnormal embryo sacs are present. However, she finds that practically every embryo sac which has been entered by a pollen tube has a series of cells derived from the endosperm nucleus, and these grapes can not therefore be considered parthenocarpic, even though seedless, but stenospermocarpic, *i.e.,* fertilization with abortion of young seeds has taken place.

Zea Mays (Corn). Mangelsdorf (96) found parthenocarpy in corn and he states that these parthenocarpic defectives contain neither endosperm nor embryo. They result from marked growth of the nucellus and pericarp due probably to the stimulus of pollination which fails to accomplish fertilization. The frequency of this type is influenced by age of silk, age of pollen and other environmental conditions.

Zizyphus Lotus. Chiarugi (18) observed specimens of this plant cultivated in the Botanic Garden at Siena, which had mature fruits of two sizes. The larger ones had seeds, whereas the small ones were without seeds. The latter were from one fourth to one third the size of the seeded ones, and the two locules sometimes showed remnants of the ovules.

Zizyphus sativus (Common Jujube). Chiarugi (19) made observations on a plant in the Botanic Garden at Florence, grown from seed introduced from China in 1894, as well as on plants propagated from those grown in Italy for several centuries. On these trees he found large seeded fruits and small parthenocarpic fruits. The latter ripened later. Parthenocarpy was quite prevalent throughout Italy and could in no way be associated with any special locality. Development of the flower and its parts as anthers and ovary were entirely normal. However, growth of the pollen tube was very slow and either did not reach the ovule at all or only after the egg was past the stage when it could be fertilized, *i.e.,* degeneration had begun. Earlier blossoms produced more parthenocarpic fruits than later flowers and the author thinks that the conditions for pollen tube growth may have been better later in the season. He found that the percentage of parthenocarpic fruits

varied from year to year. Some years it was as high as 95%, and other years only 40% of the fruits produced were parthenocarpic.

Parthenocarpy in Forest Trees. According to Hummel (77), Kurchiani found normal sized parthenocarpic fruits quite frequently in *Populus tremuloides, Salix caprea, S. aentifolia, Betula verucosa, Acer Negundo, Morus alba* and *Alnus glutinosa.* In others like *Ulmus campestris, U. montana, U. effusa, Carpinus betula* and *Fraxinus excelsa* parthenocarpic fruits were found only rarely. He also induced parthenocarpic fruit development by preventing pollination by covering the flowers with bags and emasculating those flowers that were bisexual.

Kurchiani also worked with conifers and found that in some plants it was possible to produce empty seeds by preventing pollination. As the ovary is not concerned here we do not have parthenocarpy and Kurchiani called the phenomenon parthenospermy. Plants in which he obtained parthenospermy were *Thuja occidentalis, T. orientalis, Juniperus communis, J. sabina, J. virginiana, Larix europea, Abies pectinata* and *Picea excelsa. Pinus sylvestris* failed to produce empty seeds, but he thought this may have been caused by the use of too small bags to cover the pistillate cones, as later he obtained parthenospermy with *Pinus eldarica.*

INDUCED PARTHENOCARPY

As stated in the preceding section, it is a little difficult in some instances, to decide where natural parthenocarpy ends and artificial or induced parthenocarpy begins. Parthenocarpy produced as a result of removal of most flowers from a plant, or by ringing the stem, as in grapes, for instance, has been considered natural parthenocarpy. All the investigator did was to make nutritional conditions more favorable for fruit development. On the other hand, parthenocarpy produced as a result of treatment of the style with dead pollen, extracts from pollen, chemicals or other substances as microspores from *Lycopodium,* is considered as induced parthenocarpy and will be included in this section.

Conscious attempts to produce parthenocarpic fruits date back to about the beginning of the twentieth century. Perhaps the work of Massart (97) in 1902 can be thought of as the beginning. This investigator found that dead pollen placed on the stigma of an orchid caused slight swelling of the ovary. Fitting (35, 36, 37),

Morita (100) and Laibach (86) made extracts from pollen, which when applied to the pistil also caused slight swelling of the ovary. In 1934 Yasuda (170, 171) injected a water extract of *Petunia* pollen into ovaries of *Solanum melongena* and tobacco. The ovaries did not grow into mature fruits, but the increase in size was much greater than any investigator before him had obtained. In 1935 (172) the same investigator injected an aqueous solution of cucumber pollen into cucumber ovaries, and there developed several cucumber fruits of normal size, but without seeds. Yasuda should thus be given credit for having produced the first mature fruits with a purely chemical stimulus. To be sure, he had no information as to the nature of the chemical injected and it remained for Gustafson to show that growth hormones were responsible for fruit growth. In 1936 (49) this investigator showed that the chemicals indole acetic, indole propionic, indole butyric and phenylacetic acids, when applied in lanolin paste to the style of *Lycopersicum esculentum, Petunia hybrida* and *Salpiglossus variabilis,* caused mature seedless fruits to be produced. Next year Gardner and Marth (39) and Hagemann (58) verified this with other plants, and numerous other investigators have since induced parthenocarpic fruit development with chemicals.

Methods and Chemicals Employed

By now the methods employed to produce parthenocarpic fruits with growth-promoting substances have multiplied, and it will, therefore, be profitable to present and discuss some of them.

The method as originally used by Gustafson (49) consisted of mixing the chemical with lanolin (fat from sheep wool) and this paste was applied to the stigma of the pistil or to the cut surface of the style after the stamens had been carefully removed from the bud. It was found advisable to cut the style, if long, just above the ovary to get the best effect. Laibach (87) had previously used the paste method to study the influence of growth-promoting chemicals on stem growth. This is a simple and efficient method, though a little time-consuming, and is still used extensively. Strong (136) has modified the method so that she merely cuts the bud or recently opened flower immediately above the ovary and smears the paste on this surface. This procedure requires less time, and, as Strong points out, the paste is retained not only by the style but also

by the filaments of the stamens, the corolla and even the calyx, which prevents it from coming in direct contact with the ovary. It has been found by nearly all investigators using the paste method that unless great care is exercised injury is caused to the ovary by the chemical coming in direct contact with it.

Other carriers than lanolin have also been used. In a recent bulletin, Strong (136) states that trigamine, morpholine and glycopon AA can be used instead of lanolin. They all gave a high percentage of setting and caused no injury to the fruits.

In the early experiments, the concentrations of chemical ranged from 1.0% to 5.0%, but more recent experiments have shown that lower concentrations are as effective in producing fruits, and no injury is suffered by the developing fruits. Howlett (74) recommends 0.3% of indole butyric acid as the best, and Gustafson (56) found that 0.25% and 0.5% of naphthoxy-acetic acid were both satisfactory.

Gardner and Marth (39) sprayed the pistillate flowers of *Ilex opaca* with various concentrations of several growth-promoting substances, and obtained fruit development. Satisfactory concentrations were: for indole acetic acid 0.1% to 0.04%, indole butyric acid 0.06% to 0.04%, naphthalene acetic acid 0.01% to 0.001%. The spray method would seem to be more rapid and therefore to be preferred to the paste, but Howlett (74) states that the spray method is less effective than the lanolin paste method. The writer has also found the spray method less satisfactory than the paste method. If the spray method is to be used, perhaps solvents having more adhesiveness than water could be used. Repeated spraying is necessary if buds continue to open over a period of time, because the ovary is not affected by the chemical much before anthesis. Gardner and Marth also showed that several sprayings of the same flower were more effective than one spraying.

Gardner and Marth found that watering the soil with a 0.15% indole acetic acid solution at the time the plant was in blossom produced some seedless fruits in *Ilex opaca* when the soil was watered twice. This method has not been followed by other investigators.

Yasuda (171, 172) injected an aqueous solution of pollen extract into ovaries of the egg plant, and obtained considerable growth of the ovary. Gustafson (51) used this method with known chemicals.

The chemical was injected with a small syringe through the pedicel into the ovary. Fruits of approximately normal size were produced in tobacco. In order that the injection method may be used with success, it is first necessary that the pedicel be large enough so that the needle diameter is small in proportion to the diameter of the pedicel. Secondly, the ovary and perhaps also the calyx tissue should have numerous intercellular air spaces. The injection was continued in tobacco until the ovary and calyx became dark due to the liquid filling the intercellular spaces. Only when this was done were fruits produced. Ovaries like those of cucumber, which have no intercellular spaces and are very turgid, cracked as soon as a small quantity of solution was injected, and no fruits were produced with this method in cucumber. Zimmerman and Hitchcock (173) treated flowers of *Ilex opaca* with vapors of growth substances and obtained fruits.

A number of chemicals have been used with success. Of the indole compounds, acetic, propionic and butyric acids are all active (49), but butyric is the most effective, as far as both percentage of fruits set and size of fruit produced is concerned (54, 74, 136). Their sodium, potassium, methyl and ethyl salts are also quite active (51, 173). Phenylacetic is quite active (49). Pyrrole-α-carboxylic and pyrrole-acetic acids were shown to have slight activity (51), but they are of no practical value. Of the naphthalene compounds, several show great activity: the acetic is the best known, but the oxyacetic acid (56, 174) and the substitution compound, 3,4-di-hydro-naphthalene acetic acid are also active. In fact, naphthoxy-acetic acid may turn out to be the most satisfactory of all growth-promoting substances in producing parthenocarpic fruits. Naphthalene propionic and butyric acids and the acetamide have proven to be without activity, according to unpublished data of the writer. Four-fluorene acetic acid is also active (55).

Plants Having Produced Parthenocarpic Fruits with Chemical Treatment

Antirrhinum sp. (Snapdragon). Ovaries of this plant developed into fruits one-third to one-half normal size, without any seeds, when the pistil was treated with phenylacetic, indole acetic, indole propionic or indole butyric acid (49).

Begonia sp. Treating the pistil with indole acetic or indole

butyric acid in lanolin, Gustafson (49) obtained fruits of normal
size without seeds. The fruits turned green and remained thus
until they finally dried and dropped off. Oleson (112) also ob-
tained normal sized fruits.

Capsicum annuum (Pepper). Parthenocarpic fruits have been
produced in the pepper with indole acetic, indole butyric acids (49),
the salts of indole acetic acid (51), naphthalene acetic acid (162,
163, 165), and by pollen extract from *Petunia hybrida* (50). The
parthenocarpic fruits are a little smaller than the seeded fruits and
the stylar end is frequently depressed. Internally the placental
tissue is very poorly developed, except that at times it grows into
papillae or protrusions extending into the cavity of the fruit. These
papillae may be two to three centimeters in length and up to one
centimeter in diameter. They were not found in seeded fruits.

Citrullus vulgaris (Watermelon). Wong (162, 163) was the
first to produce parthenocarpic watermelons as a result of chemical
treatment of the pistil. Gustafson (55) and Oinone (111) have also
produced such fruits. Over a period of several years, Wong ex-
perimented with a number of varieties using several growth-pro-
moting substances in various combinations. The fruits resulting
were not very numerous, but those that were formed were seed-
less and had a flesh of normal color and taste. Generally the par-
thenocarpic fruits were smaller than the seeded fruits and their
shape was sometimes triangular rather than spherical.   Both
Gustafson (55) and Wong (163) report that some varieties pro-
duced normal appearing fruits with seeds, but the seeds were devoid
of an embryo. This is definitely not stenospermocarpy because
there was no pollination or fertilization so that there could not have
been an embryo abortion. Wong had the best success with the
varieties Winter Sweet, Favorite Honey and Yellow Melon, and
he found that naphthalene acetic acid alone or in combination with
other substances was the best growth-promoting substance.   He
seems to have had better success when colchicine was added to the
naphthalene acetic acid than with naphthalene acetic acid alone or
its potassium salt.

Cucumis melo (Muskmelon). Wong (165) obtained a few fruits
in the spring of 1939. He does not give the treatment. His ex-
periments during that summer were, however, unsuccessful.
Oinone (111) had no success with muskmelon. Although they did

not produce seedless fruits, the work of Burrell and Whitaker (13) with muskmelons deserves to be mentioned. These investigators treated pollinated flowers with a 1.0% indole acetic acid-lanolin paste and the result was an increase in fruit setting of over 100%. This experiment suggests a new use for growth-promoting substances, namely, that of supplementing pollination.

Cucumis sativus (Cucumber). As has been mentioned in the preceding section, many varieties of cucumber naturally produce parthenocarpic fruits. Yasuda (172), Gustafson (50, 51, 55) and Wong (162) have produced seedless cucumbers. Yasuda injected an extract from cucumber pollen into the ovaries and obtained normal sized fruits. Gustafson produced fruits with pollen extract, indole acetic acid, its potassium salt and naphthalene acetic acid, and Wong with naphthalene acetic acid. Most of these fruits were of normal size and all were without seeds. Gustafson reports that the flesh was somewhat thicker in the parthenocarpic fruits.

Cucurbita maxima (Squash). Oinone (111), Wong (163, 165) and Gustafson (55) have produced a few seedless squashes as a result of hormone treatment of the flowers. Oinone obtained one seedless fruit of about two-thirds normal size as a result of treating emasculated flowers with indole acetic acid-lanolin paste. Buttercup squash produced an abundance of seedless fruits when flowers were treated with naphthalene acetic acid-lanolin paste (55, 165). Some of these fruits had an entirely homogeneous flesh with not even a trace of the seed cavity. Repeated attempts to produce seedless Hubbard squash was rewarded by only one medium-sized fruit (55). Other fruits grew until they were two to three times the size of the normal ovary and then dropped off.

Cucurbita moschata (Crookneck summer squash). Gustafson has repeatedly used this plant (49, 51, 55). The crookneck summer squash will develop to a considerable extent without pollination, but never into a mature fruit. Fruits produced with indole butyric acid were sometimes of normal length and seedless, but that portion of the fruit which normally produces the seeds was underdeveloped with the result that the fruit was of uniform diameter throughout its whole length. Naphthalene acetic acid (55), on the other hand, produced fruits of normal size and shape. Wong (165) also produced some of these fruits with naphthalene acetic acid. The ovaries of the crookneck summer squash are several inches long

at the time of anthesis and as a consequence lend themselves to an interesting experiment. Gustafson (51) cut off different amounts of the apex of the ovary before pollination and smeared the cut surface with indole butyric acid-lanolin paste. Even when all the ovule-bearing part was completely removed, there was an approximately normal enlargement of that part of the ovary which was left. When some of the ovule-producing part was also left, the ovary developed into a fruit nearly normal in length. This shows that a part of the ovary is capable of developing normally, even though smaller or larger quantities have been removed from it, and that the ovary does not grow as a unit, but that each portion is independent of the others, provided, of course, that it has normal access to food.

Cucurbita pepo (Pumpkin). Sereisky (131) reports production of seedless fruits when flowers were treated with a 1.0% indole acetic acid paste. The fruits were of nearly normal size with poorly developed ovules. Hubert and Maton (74a) used three varieties: Piriformis, Meloliformis and Lange Groene, and obtained a few fruits of approximately half size when the pistil was treated with a 2.0% naphthalene acetic acid-lanolin paste. Using the variety Big Tom, Gustafson (55) obtained one medium sized fruit. It is thus seen that pumpkins do not readily produce fruits parthenocarpically.

Crinum sp. Hubert and Maton (74a) obtained fruits of normal size in *Crinum americanum* and *C. longifolium* when the pistils were treated with a 2.0% naphthalene acetic acid paste. These fruits were seedless and their walls much thicker than in seeded fruits.

Cymbidium sp. Hubert and Maton (74a) sprinkled finely powdered naphthalene acetic acid on the stigma of a hybrid *Cymbidium* and there were produced seedless fruits of nearly normal size.

Datura Stramonium. Injecting 0.1% solutions and emulsions of naphthalene acetic or indole butyric acids into ovaries of *Datura Stramonium*, Van Overbeek, Conklin and Blakeslee (151) obtained fruits of almost normal appearance. Between 90% and 100% setting was obtained. The fruits contained much enlarged ovules, but though considerable growth had taken place in these ovules no embryos were found.

Digitalis purpurea. Treatment of the pistil with 1.0% indole acetic acid resulted in seedless fruits. These were shrunken, due to lack of content, but were of nearly normal size (131).

Fragaria sp. (Strawberry). Gardner and Marth (39) obtained some normal looking strawberries by spraying the flowers with 0.05% and 0.1% indole acetic acid. Never more than one fruit per inflorescens developed completely, although several may have started. Achenes developed extensively, but they were all empty. Wong (165) obtained similar results. Hunter (78) sprayed three dioecious varieties of strawberries: Louise, Portia and Simcoe, with 0.25%, 0.5% and 1.0% indole acetic acid in water or with colchicine in the same concentrations as a lanolin emulsion, and obtained a high percentage of normal fruits. Though achenes were produced they were empty. He also dusted the pistils with powdered ace-naphthene and obtained a few fruits in the Louise and Simcoe varieties. Hunter's experiments are unusual in that some flowers on the treated plants produced fruits without any treatment. He considers that perhaps there was an indirect effect of the chemical extending to parts not treated. This has not been reported by any other investigator, and as he had no control plants of these varieties there is no assurance that it was not a case of natural vegetative parthenocarpy.

Fuchsia sp. Oleson (112) produced fruits in *Fuchsia hybrida* by applying the chemical to the style or to the calyx. The latter treatment she considered more effective. Hubert and Maton (74a) injected naphthalene acetic acid (250 mg./l.) into ovaries of a variety of *Fuchsia* and obtained fruits that, as far as can be judged from their photographs, were larger than the seeded fruits. They were of normal color.

Gladiolus sp. Hagemann (58) obtained seedless fruit development in *Gladiolus* by treating the pistil with lanolin paste containing indole acetic acid. Hubert and Maton (74a) also obtained parthenocarpic fruits when they used 2.0% naphthalene acetic acid in lanolin.

Ilex opaca (Holly). By spraying pistillate flowers with indole acetic, indole propionic, indole butyric or naphthalene acetic acids, Gardner and Marth (39) caused parthenocarpic fruits to develop in holly. These fruits were of normal size and color except those stimulated by naphthalene acetic acid which remained green for a-

longer time and were somewhat larger than the seeded fruits. Gardner and Kraus (40) studied histologically the development of these parthenocarpic holly fruits and found it to parallel almost precisely that following pollination. The female gametophyte began to disintegrate about five days after treatment in spite of continued development of the integument, at least up to 302 hours. This disintegration left a cavity nearly the whole length of the enlarging ovule, but it was much smaller than the space normally occupied by the embryo. The endocarp enlarged and hardened at about the same rate in the two types of fruits and it was not possible to detect any outstanding difference in the vascular system, either in the fruit or in the pedicel. Zimmerman, Hitchcock and Wilcoxon (173) have obtained parthenocarpic fruits in *Ilex opaca* by exposing the flowers to vapors of growth substances.

Luffa cylindrica. Sereisky (131) found that when he treated the pistils of flowers that had just opened with a 1.0% indole acetic acid-lanolin paste he was able to produce seedless fruits that were nearly as large as the seeded fruits, but when flowers either older or younger were treated, the results were very poor. The parthenocarpic fruits were more slender than the seeded fruits. In some the seed coats developed considerably, but at no time were there any embryos.

Lycopersicum esculentum (Tomato). *Lycopersicum esculentum* was the first fruit to be produced artificially with known chemicals, and because it is of commercial importance it has been used by a number of investigators (49, 73, 80, 111, 137, 174). In 1936 Gustafson (49) was able to obtain fruits of normal appearance by treating the cut style with phenylacetic acid, indole acetic, indole propionic or indole butyric acids. These fruits were seedless and as a rule the locules were reduced in size; in fact, they were sometimes entirely absent. In some fruits the ovules were quite well developed, in others only the chalaza showed any growth, and, in still others, the ovules were not even visible. In general, it can be said that the pulp increased at the expense of the gelatinous material, which decreased in quantity. The taste was essentially normal. Janes (80) has made a chemical analysis of parthenocarpic and seeded John Baer tomatoes, and he found that the former had a higher sugar and lower acid content than the latter. The parthenocarpic fruits had also a slightly higher per cent dry material

than the seeded fruits. Howlett (74), who has been interested in the tomato from the practical standpoint, has reported that fruit produced with indole butyric acid is larger than seeded fruit. Gustafson (54) and Strong (137) have found the same to be true. The per cent of setting is at least as high with chemical treatment as with pollination (54, 56, 74, 137). Both Howlett and Strong have been interested in developing simple methods of producing fruits in tomato with chemicals that could be used by untrained people, because during winter tomatoes do not produce much pollen, and if setting could be increased by chemical treatment of the flower, it would be of considerable value commercially. Zimmerman and Hitchcock (174) sprayed tomato flowers with β-naphthoxyacetic acid and obtained seedless fruits. Tatarintsev (143) obtained some very interesting and unaccountable results. In his experiments he used the varieties Ficarazzi, Peach, King Humbert, Prince Borguese and Pear-shaped Yellow, and also *L. pimpernellifolium*. His treatments consisted of pollination with potato pollen, dusting stigma with *Lycopodium* spores, or dry soil, irritating the stigma with a pair of forceps or a paint brush. Considering the experiments as a whole, we find that he obtained 30% setting when flowers were emasculated and untreated, 60% with potato pollen, 35% with *Lycopodium* spores, 40% with dry soil treatment, 50% with forcep irritation of the stigma and 35% with brush irritation. As a whole, the plants showed a tendency toward natural parthenocarpy, and only the potato pollen and irritation with forceps produced a percentage setting much greater than natural. However, when one considers individual varieties this explanation does not hold. *L. pimpernellifolium* produced no fruits when flowers were emasculated without further treatment, showing complete absence of natural parthenocarpy. Yet 20 fruits were produced with potato pollen, 15 with *Lycopodium* spores, 50 with dry earth, 40 with forceps irritation and 55 with brush irritation. This is a very remarkable experiment and there seems to be no explanation for these results. Most of the fruits were usually lacking in the gelatinous material ordinarily found in the locules; the locules were empty or very much underdeveloped.

Melandrium dioicum. Van Overbeek, Conklin and Blakeslee (151) obtained mature fruits of nearly normal size without seeds when the ovaries were injected with 0.1% naphthalene acetic acid.

Indole butyric acid of the same concentration gave nearly as good results, whereas indole acetic acid failed to produce any fruits. External application at the base of the flower or on the outside of the ovary also produced fruits, but unlike those fruits produced by injection the ovules and placentae enlarged only slightly. Although the ovules in the fruits produced by injection enlarged very much, there were no embryos observed.

Nicotiana sp. Yasuda (171) obtained considerable growth in tobacco by injecting an extract of *Petunia* pollen into the ovary; and by injecting potassium indole acetate, in concentration of 1 : 500, into the ovary of Maryland Mammoth variety, Gustafson (51) obtained fruits that were very nearly normal in size, but without seeds. It was found that the ovaries treated with the chemical grew more rapidly than those stimulated by pollination during early development of the fruit, but the rate slowed down so that the final size of the fruit was somewhat less than that of the seeded fruits. The chemical applied in lanolin paste to the cut style was not so effective as injection into the ovary.

Oncidium longipes. Hubert and Maton (74a) produced parthenocarpic fruits in this plant by applying crystals of naphthalene acetic acid to the pistil.

Petunia hybrida. Gustafson (49) had an abundance of ovaries develop into fruits as large, if not larger than, seeded fruits, when the cut style was treated with indole acetic, propionic or butyric acid in lanolin. While as large as the seeded ones these fruits were wrinkled and empty. The rate of development was about normal. Even varieties that produced no fruits with pollination were caused to produce fruits with chemical treatment.

Primula Hortensis. Oleson (112) induced parthenocarpic fruit development in this plant by treating the pistil with growth-promoting substances in lanolin. The fruits were normal in size.

Pyrus communis (Pear). Sereisky (130) obtained seedless fruits in the variety Caucasicus when the flowers were treated with indole acetic acid in concentrations 0.1%, 0.5% or 1.0%. They were nearly as large as seeded fruits; the core developed to some extent but the ovules did not. Sereisky's experiments are of special interest because everyone else who has tried to induce parthenocarpy in pomaceous fruits has failed. There is, however, no doubt about his results, as his photographs are clear, the fruits show no seeds and very little core is in evidence.

Salpiglossus variabilis. Treating the pistil with indole acetic, propionic or butyric acid, Gustafson (49) produced parthenocarpic fruits of large size, but wrinkled and empty. The percentage of setting was fully as large as with pollination.

Solanum melongena (Egg Plant). Egg plant was one of the first plants to be used successfully in experiments on induced parthenocarpy with growth-promoting substances. Yasuda (170) obtained small fruits by injecting *Petunia* pollen extract into the ovary of the emasculated flower. Gustafson (50) has repeated this experiment by applying the extract in lanolin paste to the style. The latter (49, 51) has also obtained parthenocarpic fruits by applying indole butyric acid, potassium indole acetate, or pyrrole-α-carboxylic acid in lanolin to the stigma. When time for proper development was ample, these grew into normal fruits, though somewhat smaller than the seeded ones. Control flowers without treatment did not produce fruits. Oinone (111) has produced seedless fruits of egg plants with indole acetic acid applied to the stigma or cut style. His fruits ranged in size from 11 to 15 cm. as compared with 17 cm. for the seeded fruits. He reports that "the softness of their flesh was such that it melted in the mouth when it was cooked". He does not mention percentage of setting, but makes the statement that the production of parthenocarpic fruits with heteroauxin was easy.

Vitis vinifera (Grape). Oinone (111) produced seedless fruits on five year-old grape vines (Berlandieri × Riparia) with oestrone and indole acetic acid. They were smaller than seeded fruits and those produced by oestrone were the smallest.

DISCUSSION

No general statement covering a comparison of parthenocarpic and seeded fruits can be made. The nearest approach to a general statement is that as a rule parthenocarpic fruits are smaller, but even to this there are exceptions. Hume (76) found that in *Diospyros Kaki* the seedless fruits or fruits with one or two seeds were larger than the seeded ones. Howlett (74), Gustafson (54, 56) and Strong (136) have found that parthenocarpic tomatoes produced by indole butyric, naphthalene acetic and naphthoxyacetic acids are larger than the seeded.

Seedless pears have been found by some investigators (31, 32,

72, 121, 154) to be somewhat more slender, especially in that part of the fruit where seeds are usually formed, than seeded fruits. Seedless apples are longer and have a greater depression at the calyx end than the seeded fruits (31, 32). Parthenocarpic peppers are short and have a prominent depression at the apical end (49, 80). Crookneck summer squashes induced with indole bu ... acid are more slender than normal fruits (49), whereas those ... duced by naphthalene acetic acid are normal in appearance (Thus we see that the shape of parthenocarpic fruits is sometimes different from that of corresponding seeded fruits, and most of this difference is caused by lack of enlargement of that part of the fruit in which the seeds should be formed.

Cytological or histological studies have been made in only a few instances during development of parthenocarpic fruits. Salacolu (124) states that in the parthenocarpic fruits of *Brassica Oleracea, Lonicera Caprifolium, Papaver Rhoeas, Lilium candidum, Lunaris biennis, Paeonia officinalis* and *Rhododenron ponticum* the cells were as numerous as but smaller than in the seeded fruits, and the vascular system was poorly developed. Gardner and Kraus (40), on the other hand, found in *Ilex opaca* that the vascular system was as well developed in the parthenocarpic as in the seeded fruits, and they found no other difference in development, except that no embryos were formed.

Taste and chemical differences have also been investigated. Some investigators, as Ewert and Müller-Thurgau, thought that seedless fruits had the better taste, whereas Wölfert (161) states that some seedless pears which were sent him had a taste inferior to that of seeded fruits, and Nixon (109) declares that seedless dates are coarse and stringy and lack flavor. That there may be a difference in taste is brought out by chemical analysis. Müller-Thurgau (101) found that seedless grapes had a higher percentage of sugar and a lower percentage of acid than the seeded fruits. Ewert (33) noted that parthenocarpic gooseberries contained a higher percentage of both sugar and acid. Condit (25) reports that in several varieties of figs the uncaprified (seedless) fruits had a higher sugar content and dry weight than the caprified. Hume (76) and Condit (24) have found that the seedless fruits of *Diospyros Kaki* (oriental persimmon) are light colored and quite astringent until soft and ripe, whereas the seeded fruits are dark

and non-astringent, even when hard. Reinecke (120, 121) found that seedless Kiefer pears stored for several weeks had a lower sugar and acid content and a shorter storage life.

The most detailed chemical analysis of parthenocarpic fruits is that made by Janes (80) on tomatoes and peppers. This investigator found that the percentage of titratable acidity was higher in seeded fruits, whereas the opposite was true for sugar. Distribution of acid was quite uniform in the parthenocarpic fruits, but in the seeded fruits there was less acid in the pericarp and more in the locules than in the corresponding parts of the parthenocarpic fruits. Parthenocarpic peppers had a higher percentage of dry weight and also a slightly higher percentage of nitrogen in the pericarp. There was no difference in acid or sugar content.

Again, as to the time required for ripening of seedless fruits as compared with seeded fruits, there is no unanimity. Ewert (33, 34) found that seedless gooseberries and apples ripened earlier than the corresponding seeded fruits. According to Woodburn (166), the seedless fruits of *Diospyros virginiana* ripened earlier, and Strong (136) reports the same for tomatoes. On the other hand, Wölfert (161) states that seedless fruits of *Solanum nigrum* are slower to ripen than seeded fruits. Chiarugi (19) found the same for *Zizyphus sativus* and so did Nixon (109) for the date. Most authors make no statement as to time of ripening, and from the above it is evident that it is a matter of species.

It is evident to anyone even slightly familiar with the subject that various conditions exert quite an influence upon the production or non-production of parthenocarpic fruits. Climate seems to be such a factor. Wong (162) states that usually the Navel orange and the Marsh grapefruit are seedless in California but seeded in Florida. Grapefruit produces seeds also in Trinidad. In a private communication, Condit says: "In Texas the Brunswick (Magnolia) fig is almost entirely parthenocarpic; in California this variety drops its young immature fruits in considerable number". He goes on to point out that even the same branch be
·es differently during different parts of the same growing season.
·.he case of White San Pedro, Gentile, Dauphine and certain
ier varieties of this so-called San Pedro type, is especially interting. The first-crop figs are completely parthenocarpic, the
econd-crop fruits on the same branch in the same season are com-

pletely non-parthenocarpic". Reinecke (120) finds that many varieties of pears and apples as a rule produce only seeded fruits in the United States while they produce parthenocarpic fruits in South Africa.   Waite also points out differences in the behavior of apples in New York State and California.

The vigor and general nutritional level of a plant has been found to be an important factor in the development of parthenocarpic fruits.   Ringing of the stem to keep a larger amount of the photosynthate in the branches has been found very beneficial in production of parthenocarpic fruits.   Thus, Müller-Thurgau (102) got a higher percentage of seedless grapes when the canes were ringed. Paulsen and Berna (114), Jacob (79), Olmo (113) and Pearson (116) have noted the same.

Munson, Ewert, Müller-Thurgau and others have noted that unless a tree is very vigorous, no apples or pears without seeds will be formed, except when pollination is completely prevented, and Wölfert found that only by removing all but one flower from each plant was he able to produce parthenocarpic fruits in *Solanum nigrum*.   Höstermann found that as his pumpkin plants became more vigorous they produced seedless fruits.   Ewert found that apple flowers in the center of a cluster, with large ovaries, were much more likely to produce fruits without pollination than the smaller ones on the outside, and his procedure was to remove all but a few of the central flowers.

From the above it is obvious that parthenocarpic fruits require more favorable conditions for development than do seeded fruits, and sometimes the seedless fruits cannot compete with the seeded. Some investigators have suggested that parthenocarpy in a species is a sign of hybrid origin.   Others deny this.   It is very possible that the vigor of hybrids may be conducive to parthenocarpy, and for that reason many hybrids may produce parthenocarpic fruits, not because they are hybrids but because they are vigorous.   This coupled with the fact that many hybrids are sterile may be the explanation of parthenocarpy frequently appearing in plants of hybrid origin.

Many investigators mention the relation between number of seeds and size of fruit and all of them are agreed that the seeds exert an influence.   Some consider that the seeds "draw" food into the fruit.   Heinicke made some very interesting observations on

apples. He found that osmotic pressure of expressed sap of the fruits increased with the number of seeds. He also noted that leaves of apple branches with fruits covered with vaseline wilted more slowly than branches without fruits and that branches with fruits having many seeds wilted sooner than those with fruits having few seeds. The branches were severed from the tree. He also found that on thick spurs, with a greater vascular system, fruits with few seeds were able to develop, whereas on thin branches only fruits with many seeds developed. From these and other experiments it is assumed that seeds aid in conduction of materials into fruits, and fruits without seeds have greater difficulty in obtaining needed material and will therefore be able to develop only when nutrition is high or when there is little competition.

A very special external condition responsible for parthenocarpy has been observed by several investigators. Gardner (43) found in his work on self and inter-sterility in cherries that plants heavily infested with aphids sometimes produced fruits even though under normal conditions they were sterile. Many of these fruits dropped while still young, but others remained on the trees till mature. The stones developed to nearly full size, but as a rule they contained no seeds. Kraus (84) found that in self-sterile apples it is usually possible to produce fruits if the blossoms are infested with aphids. Heinicke (65) also found that if apple buds and blossoms are heavily infested with aphids, many flowers even though not pollinated will produce fruits which, however, are small and deformed. The fruits remained on the trees until ripe.

Treub (149) found that closed flowers of the orchid *Liparis latifolia* developed into fruits, and on examination he found that the ovaries contained several larvae. Noll (110) noted blossoms of *Cytisus Adami* growing into fruits without pollination as a result of insect larvae developing in the ovaries. And Fitting (35) states that Forbes observed larvae in the ovary of the orchid *Calanthe* sp. and that these ovaries developed into fruits. Höstermann (72) observed some rather abnormal parthenocarpic pears and considered that they had been produced as a result of development of larvae of the pear gall gnat (*Contarinia pirivora*). These results are not surprising in view of present day knowledge of hormones. La Rue (88) made the discovery that the feces of leaf miners contained growth hormones, and Link (91a) has reported that aphids are a regular storage house for auxin.

We have thus far considered some of the conditions which favor, or are associated with, formation of parthenocarpic fruits, and it now becomes necessary to attempt an explanation for their development. In attempting this it is necessary first to consider normal fruit development. Enumerating, in sequence, the happenings, we have pollination, pollen tube development, fertilization, embryo development, and it is usually stated that the ovary begins to enlarge as a result of embryo growth. However, this is probably not entirely true. Hildebrand (67) was of the opinion that pollen has two separate actions. One is fertilization of the egg, the other its influence upon the ovary in causing it to begin to grow into a fruit. Continued development of the ovary may depend upon fertilization and growth of the embryo, because as a rule the ovary soon ceases to grow if embryos are not formed. Closely associated with commencement of ovary growth is prevention of an absciss layer between it and the pedicel. Prevention of this abscission layer may even be the first step in fruit formation, and we frequently find that this is as far as fruit growth goes. Abscission may take place any time between withering of the flower and maturation of the fruit. To horticulturists it is a well known fact that many fruits started on their development drop long before maturity. Dorsey (28a) and others associate this with lack of seed development. That the dropping of immature fruits is associated with growth hormones is evident from a number of experiments. Gardner, Marth and Batjer (41, 42) and others have shown that spraying nearly mature apples with growth-promoting substances (auxins) prevents them from dropping. Laibach (87), La Rue (88) and Myers (105) have shown that if the debladed petioles of many plants are treated with auxins they will not drop nearly so soon as those not so treated. There is no reason to suppose that formation of the abscission layer in leaves and fruits is not brought about in the same way. Heinicke (65) found that when he removed apple fruit from the peduncle, the latter dropped very soon. While this author does not speak of hormones, there is no doubt that this is proof that hormones are responsible and that they are produced in the fruits. Continued growth of the fruit is influenced by the seeds. Bailey, Ewert, Müller-Thurgau, Heinicke and many others have contributed information which shows that there is a relation between the number of seeds and fruit size. Further evidence that seeds are important

in fruit growth has been furnished by Dollfus (28) and Sereisky (130). The former found that when the central part of a fruit, composed of ovules, placentae, *etc.,* was removed, the fruit stopped growing, but continued growth was obtained when lanolin paste containing auxin was placed in the cavity. Sereisky found essentially the same thing in pears and apples. Dollfus (28), Meyer (98) and Gustafson (53) have shown that the seeds of developing fruits are rich in growth hormones. We thus get the picture that growth hormones in ovules and seeds are necessary to development of seeded fruits, and we may then pertinently ask, where do seedless fruits get their hormone?

In earlier pages it has frequently been mentioned that seedless fruits have developed as a result of crossing one plant with another variety, species or genus. Hildebrand (68) observed that many times in cross pollinations the pollen tubes grew only slightly, and that though they did not reach the ovary, fruits were nevertheless formed but were seedless or with empty seeds. Müller-Thurgau (101) reported similar observations. Yasuda has made many experiments to determine the influence of pollination upon fruit formation. He found that egg plant flowers pollinated with *Petunia violacea* grew into seedless fruits (170). Ovaries of *Capsicum* sp. were stimulated to grow into fruits when the flowers were pollinated with a species of *Physalis* (168a). Pollination of *Nicotiana* sp. with pollen from *Petunia violacea* resulted in seedless fruits, but pollen of egg plant had no such stimulating effect (171). Pollen tubes of *Petunia* grew faster than those of egg plant, in the style of *Nicotiana*. The latter pollen tubes made only slight growth. Yasuda found that when cucumbers were pollinated with their own immature or old pollen or pollen from other cucurbitaceous plants, fruits developed sometimes, and these were always without seeds. When fruits were formed, pollen tubes had also made some growth, and when pollen tubes were not formed, neither were there any fruits. From these and other experiments, Yasuda began to realize the importance of pollen tube penetration into the ovary, and he contrived experiments in which he cut off the style at different time periods after pollination to determine how close to the ovary the pollen tubes had to come in order that the ovary would be stimulated to develop. In one experiment (172a) he pollinated flowers of egg plants and cucumbers with their own viable pollen and cut

the style immediately above the ovary at different time intervals after pollination. From these experiments he found that the pollen tubes had to reach the base of the style or the top of the ovary before the ovary would be stimulated sufficiently to grow into a fruit.

We thus have ample evidence that pollen tube penetration into the style or ovary is sufficient, without fertilization, to cause fruit development in many plants. It would seem logical to assume that pollen tubes transfer to the ovary a chemical or chemicals which initiate ovary development. From the fact that growth-promoting chemicals, when applied to the pistil, also cause fruit development, one might suspect that pollen tubes transfer growth hormones to the ovary, which causes it to commence growth. Yasuda (170, 171) and Gustafson (50) have shown that pollen extracts, when applied to the pistil of a flower, may cause it to develop into a fruit. Laibach (86) and Thimann (144) have shown that pollen contains growth hormones.

Growth hormones undoubtedly play a part in fruit production; whether it is a direct or indirect role is difficult to say. The writer believes that the action is at least partly direct. The pollen tubes bring growth hormones into the ovary and the first effect of this hormone would be to prevent abscission of the flower. The ovary would thus remain in contact with the outside food supply. It is well known that not all flowers that remain on a plant develop into fruits; therefore there is more to fruit development than access of an ovary to a supply of food. As mentioned previously, seeds are very important and even necessary in some instances for growth of fruit. Ovules and seeds have been shown (28, 98, 52, 53) to contain growth hormones. How does this growth hormone or that which is added artificially act to bring about enlargement of the ovary into a fruit? The primary function of a growth hormone is to cause cell enlargement. Is that the only function of the growth hormone, or is it also responsible for transfer of building materials into the developing organ? We have not enough information at present to answer these questions. We can, however, consider that developing ovules produce or concentrate growth hormones in the fruit which are directly, indirectly, or both, responsible for continued growth of the ovary into a mature fruit. Gustafson (52) has shown that plants which normally produce parthenocarpic fruits have a higher auxin content in the ovaries than do varieties

that produce fruits only as a result of pollination and fertilization. The same author has also shown that artificially produced parthenocarpic tomatoes have considerable growth hormones in them, though it is less than in the normal seeded fruits.

We thus could formulate the hypothesis that some plants under some conditions produce enough growth hormone so that with or without pollination, as the case may be, they are able to prevent the absciss layer from being formed in the pedicel, and that under favorable nutritive conditions and with a minimum of competition, they are further able to transport the necessary food and bring about enlargement of the cells in the ovary to produce mature fruits without seeds, whereas other plants are unable to do this.

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TAXONOMY AND PHYLOGENY

W. B. TURRILL

Royal Botanic Gardens, Kew, Surrey, England

PART III

CONTENTS

CLASSIFICATION AND PHYLOGENY IN THE MAJOR GROUPS

The early systematists understood very little of the nature of somatic morphology, including anatomy, and of reproduction of the cryptogams. They usually made a few, often not more than six to ten, groups equivalent to what we should now term families in the seed-bearing plants for all the cryptogams. Linnaeus described in 1753 about 600 species of cryptogams as against 5322 spermatophytes. Even so late as 1847, Lindley included all cryptogams (Thallogens and Acrogens) in 25 natural orders as contrasted with 278 for the seed-plants. The increase in our knowledge and understanding of cryptogams in the last 80 years is far more striking than it is for phanerogams. It is not proposed here either to trace the history of our knowledge of cryptogams and phanerogams or to give details of systems proposed for them. Within every group, however, there are problems which have a wide general bearing on our main theme and others which, though peculiar, or with our

present knowledge apparently peculiar, to the group, must be considered unless the problems of both classification and phylogeny are to be unduly simplified. Here only a few references are given for each group, and these are mainly to recent publications which themselves contain numerous references to earlier literature. Lotsy (272) gives excellent accounts of phylogenetic ideas in the various groups up to the dates of publication of his volumes. Scott (385), Seward (388), Zimmermann (506) and Darrah (124) deal with and give literature for the palaeobotanical aspects. The problems considered below have been highly selected for the special purposes indicated, and no pretence is made to deal adequately with the classification or the possible phylogeny on any of the groups. Considerations of space forbid such an attempt in this paper.

It is interesting to note that even in the viruses the need for classification has already been felt (252, 38, 225, 460 a).

Bacteria

Classification of bacteria is a particularly difficult subject, owing to the very few morphological criteria available in these microscopic unicellular organisms which are without definable nuclei and have no sexual reproduction. Biochemical and biophysical reactions have to be used for distinguishing certain species. Broad groups, usually named as genera, can be distinguished, and "a committee of American bacteriologists have lately devised a system of classification which, though not yet universally accepted, is receiving steadily increasing support" (155). Metcalfe has given a summary of newly proposed systems of classification of bacteria (300). Nothing is known with certainty as to the origin of bacteria. Statements that they represent the most primitive form of existing organisms are counterbalanced by conclusions that they are degenerate forms produced by reduction from some other group or groups, *e.g.*, Cyanophyceae (436). As Zimmermann (506) points out, to distinguish bacteria from Cyanophyceae in the fossil condition is difficult and at times impossible. A summary of their reported geological history is given by Pia (338). It is most unlikely that the bacteria are in any main line of plant evolution.

Algae (sensu lato)

Smith (402), after commenting on the inadequacy of a classification of thallophytes based upon either the vegetative structure or

the methods of reproduction, says: "It has become increasingly clear during the past quarter century that the morphology and the physiology of the individual cells are the fundamental bases upon which the algae must be classified. This evidence shows that there are several series among the algae, each of which has cells with certain distinctive physiological and morphological traits. Chief among the morphological characteristics is the structure of the motile cell, and for most of the series among the algae there is a striking constancy in its organization, especially with respect to the number, arrangement, and relative length of the flagella. On the physiological side there is, throughout each series, a constancy in the pigments present in the plastids and a constancy in the chemical nature of the food reserves accumulating through photosynthetic activity. . . . This constancy with which the morphological and physiological characteristics obtain in each series and the marked differences between the various series (Chlorophyta, Cyanophyta, Phaeophyta, *etc.*), suggest very strongly that the various major groups of algae have but little in common with one another".

Earlier views on the relationship of the principal groups of algae to the flagellates are adequately summarized by Cavers (82). Some of these views are not now accepted. For more recent opinions reference should be made to the works of Fritsch (150, 151) and the literature lists given by him. Macfarlane (278) concludes that "the most ancient, and in structure most primitive plants are probably the Schizophyceae (Cyanophyceae), which may have originated during the Mid Archaean or Proterozoic epoch, and were probably active agents, then, as now, in forming the siliceous and calcareous beds encountered in the strata of that period". Many of the Cyanophyceae are thermo-resistant. "The thermophilic bacteria also represent an ancient series, either derived from the Schizophyceae by disappearance of the synthesizing cell pigments, or arising independently by utilization of energy from sulphur or siliceous compounds".

Fritsch (151) shows that the old distinction between algae and flagellates can no longer be maintained. He recognizes eleven classes of algae and says that at the present time it is perhaps best to regard each of these as a separate evolutionary series until a clear relation to the others is indubitably established. Excepting for the larger calcified forms, the study of fossil algae is "confronted with

the difficulty that, in the absence of any data as to the nature of the cell-contents or of the methods of reproduction, reference to any particular class is well-nigh impossible". The fossil history of the algae is summarized by Pia (337) and by Zimmermann (506). Tilden (436) considers "a parallel development of types, rather than the usually accepted tree-like form, is to be regarded as the course followed in the evolution of the different phyla of marine algae". In a later work (437) she boldly gives a chart with algal periods inserted in the geological time table. Fritsch gives very striking tables showing parallelism in a large number of characters, sometimes involving a majority of the groups. A further difficulty in tracing phylogeny in the algae is that simpler forms may be reduced from forms with a higher somatic development or may be primitively simple, and it is often impossible to be certain into which category they should be placed. That a number of quite distinct lines can be traced upwards from the Flagellata is now well established on grounds that make it very probable that the algae are highly polyphyletic.

The relationships of algae to other groups of plants is a very speculative subject. Their possible connections with fungi are discussed under that group. As an example of the very diverse views expressed on the phylogeny of a large group, it is of interest to note that Church (94) has even suggested that the simple freshwater algae are reduced from more highly elaborated land plants. Fritsch (150) does not accept this as a possibility. He believes "that the more highly elaborated Green Alga became a land-plant, the early forms of which are perhaps yet to be disclosed by palaeontological research. . . . A more intensive investigation of the Chaetophorales, and in particular of the terrestrial Trentepohliaceae, than has hitherto been undertaken may well afford data bearing upon the relation of the Isokontae to higher plants". Church's (94, 95) accounts of the subaerial transmigration of the hypothetical Thalassiophyta has been so often discussed in the last two decades that it is not necessary here to give details of his theory. His account of the somatic organization of the Phaeophyceae is a particularly valuable summary of knowledge on the vegetative phase of this large group.

The Charales (Charophyta of some authors) are considered by Fritsch (151) as an order of the Chlorophyceae. They have been

very variously placed by different botanists—in the algae as a distinct group of cryptogams, or even in the Bryophyta. However they be classified, their relationships are vague and they remain an isolated and not a synthetic group, in the sense that they have a great many characters that are peculiar to the group and few that are homologous with or analogous to those of other groups. It is only by weighting a few characters that they can be classed with the algae. Charophytes are abundant as fossils in certain strata. Harris (203) has described in detail those from the British Purbeck rocks (Upper Jurassic), gives the evidence that plants with the vegetative organization of *Chara,* the most elaborate of the modern forms, already grew in Upper Jurassic times, and concludes that "there is nothing to link this class up with any other group of algae".

Fungi (sensu lato)

The large and heterogeneous assemblage of colourless parasites and saprophytes included under this class-term is divided into main groups chiefly on their reproductive organs. The question of their origin "is highly controversial and opinion is divided as to whether they arose from the protozoa or whether they had either a monophyletic or polyphyletic origin among the algae. If they arose from protozoa, they should be put in one or more divisions coordinate in rank with the various algal divisions; if they arose from the algae, they should be placed as classes of one or more of the algal divisions" (402). This statement of Smith's is an interesting example of the principles underlying classification based, as far as possible, on conclusions as to phylogeny. To classify the fungi among the algae would scarcely please the mycologists, and it would be most inconvenient for the important practical purposes of mycology. There seems a general tendency to consider the later alternative as a very possible phylogeny—that fungi can be arranged in series leading upwards from colourless protista. This would mean that the fungi are not degenerate forms of autotrophic green plants but plants which have evolved in connection with saprophytism and parasitism.

Brooks (67) holds that the fungi are "a monophyletic group, established in far distant times from protist organisms, which have evolved along their own lines just like any other group of plants or animals". Ramsbottom (347) discusses certain general prin-

ciples of taxonomy and also some of the special difficulties met with in the fungi. He has also (in 236) pointed out that while the main problems of classification are the same in mycology as in a taxonomic study of phanerogams, the methods used to solve them are different in important respects—especially in their modern developments. More or less peculiar to fungi are cytological difficulties due to small nuclei, the possible rarity of hybridization, the difficulty of determining polyploidy, the saprophytic or parasitic mode of life of all members of the group, saltation (a term which probably covers more than one phenomenon), the dual phenomenon, extensive heterospory in some groups, heterothallism, hyphal anastomoses, and the absence of known sexual phases in the life-histories of the great group of Fungi Imperfecti. In another publication Ramsbottom (348) refers more fully to phylogeny and concludes: "There is, as one would expect, some divergence of view, but the one which is becoming more generally accepted is that fungi were originally derived from colourless flagellates related to the coloured forms which gave origin to the algae; that they have evolved by perfecting the parasitic and saprophytic habit; and that the principal groups of fungi are more closely related to each other than they are to any group of algae".

Gauman and Dodge (159) give a phylogenetic diagram of the fungi in illustration of their extreme statement: "All true fungi are derived from green algae in monophyletic line". It is difficult to interpret the word monophyletic here, except in the sense that all true fungi, *i.e.*, excluding Myxomycetes and Archimycetes, are derived from the group Chlorophyceae.

Linder (268) discusses, with many references, the phylogeny of the Basidiomycetes and says it is possible to observe that there has been a general trend that has led from the Uredinales to the higher groups of the Basidiomycetes.

Martin (295) considers that the Myxomycetes (Mycetozoa) "constitute a specialized group whose affinities are to be sought amongst the colorless flagellates". Their specialization has led no further and they represent the end of one developmental series. "The Phycomycetes, on the other hand, present a reasonably continuous series which, through their higher forms, may well have given rise to the Ascomycetes and, through them, to the Basidiomycetes".

Various problems of relationship in the fungi are discussed by Gwynne-Vaughan (177) and Gwynne-Vaughan and Barnes (178).

Lichenes

The dual nature of the lichen body as a composition of a fungus and an alga is now generally accepted. Moreau (310) gives in some detail the various views regarding this symbiosis and the observations and experiments on which it is based. In place of the frequently expressed view of a harmonious and mutually advantageous symbiosis he believes the relationship between the two . components to be rather of the nature of a truce or armistice.

The fact that the algal component and, in a smaller number of lichens, the fungal component also, can be isolated and determined as an independent organism, raises interesting and peculiar problems of taxonomy and phylogeny. One theoretically extremely natural, or at least rational, scheme would be for every lichen to receive a name which would indicate the two organisms taking part in its formation, with a classification based on one of the components and another based on the other, but this could, at present, be done only for a limited number of fungal components. It is much more convenient to give independent generic and specific names based on the characters of the dual organisms themselves, while the primary (higher) divisions are based on the fungal partner which alone produces sexual organs, whether functional or not. Moreover, Tobler (444) concludes: "that more than ever modern researches showed the specific and independent character of these organisms from the morphological, as well as from the physiological and phylogenetic points of view".

Smith (400), in her chapter on phylogeny of the lichens, has many interesting remarks, a few of which may be quoted or paraphrased here. "Though lichens are very old members of the vegetable kingdom, as symbiotic plants they yet date necessarily from a time subsequent to the evolution of their component symbionts. Phylogeny of lichens begins with symbionts". The associated algae, Chlorophyceae and Myxophyceae, had become aerial before their association with fungi to form lichens, and "it is possible to refer them to the genus or sometimes even to the species of free-living forms. The fungus hyphae have combined with a considerable number of different algae, so that, even as regards the algal

symbiont, lichens are truly polyphyletic in origin". Since the fungus is the dominant partner, "the principal line of development must be traced through it". Both Basidiomycetes and Ascomycetes form with various algae the lichen families. Ecologically and biochemically by the production of lichen-acids excreted by the fungus but peculiar to lichens, lichens are quite distinct from algae and fungi, and to this extent any phylogenetic classification would fail to show their essential and actual autonomy. The numerous families and genera of algae (of the Myxophyceae and Chlorophyceae) taking part in lichen formation are listed by Smith. The fungal relationships are indicated in the following classification:

    a. Hymenolichens (probably to Thelephoraceae).
    b. Ascolichens.

        1. Pyrenocarpineae }
        2. Coniocarpineae   } to Pyrenomycetes.
        3. Graphidineae   to Hysteriaceae.
        4. Cyclocarpineae   to Discomycetes.

The great bulk of lichens are ascolichens, and even for these "the theory of a polyphyletic origin for the different series seems to be unassailable. At the same time, there is no evidence to show in which series symbiosis started first". Probable or possible progressive series can be traced in the different groups, and possible courses of development for the different organs (organogenesis).

Attention should be called to the work of Chodat and others (92) in which, by pure culture methods, it has been shown that the building up of a lichen is by no means haphazard, but that it involves the coming together of a definite species of fungus with a particular type or elementary species of alga, and only with this one combination will a lichen of a particular species be synthesized.

Bryophyta

This is one of the most interesting of all plant groups from the standpoint of taxonomy and phylogeny. Abundant material is easily collected and preserved. The group itself is well demarcated by the conspicuous alternation of generations, with the gametophyte the dominant generation on which the sporophyte is largely

or entirely dependent. Relationships with the Pteridophyta, on the one hand, and with the algae, on the other, are highly hypothetical. The fossil history of both Hepaticae and Musci sheds little light on their ancestry or other relationships, though Walton (476), after a careful examination of material of Carboniferous age, says that "it removes all doubt as to the presence of Liverworts of a comparatively high degree of specialization in the Palaeozoic, and effectively negatives any suggestion that the Liverworts are a 'recently' evolved group". For the mosses Walton concludes on fossil evidence that "there seems to be little reason to doubt the presence of Mosses at least as far back as the Stephanian (Upper Carboniferous), but what their relationships were to the divisions of the living Mosses cannot yet be decided". Harris (202) refers *Naiadita,* of which he examined and very fully described abundant reproducing material of Rhaetic age, tentatively to the Sphaerocarpales near the Riellaceae.

For the major divisions of the Bryophyta, as usually accepted, there appears to be a considerable degree of genetic and phylogenetic independence of the sporophyte and gametophyte. Thus, to take only one example, the vegetative structure of the gametophyte of *Anthoceros* is simple and has what are usually considered algal characters. On the other hand, in its sporophyte it shows a more complicated structure with characters at least reminiscent of Pteridophyta. In both liverworts and mosses, for both sporophyte and gametophyte, there are numerous and striking examples of convergence of characters which must be regarded as homoplastic on our present knowledge. It is not difficult to arrange series which can be postulated as morphogenetic (progression series), but, as Harris has said, "In studying phylogeny it should be obvious that the morphological series may often not represent evolutionary series at all, and, even if they do, it is a matter of extreme difficulty to decide which end of such a series is primitive".

Various writers on bryophytic phylogeny have considered the liverworts as more primitive than the mosses and have held that they arose from a simple dorsiventral type of gametophyte associated with a very simple sporophyte. Cavers (81), for instance, postulates a Sphaero-Riccia ancestor which gave rise to the Marchantiales and, independently, to the remainder of the Hepaticae

and through them the Musci. Campbell (78, 79) accepts a very similar view for the main mass of the liverworts but would give the Anthocerotales a much more independent position and origin. Mielinski (306), as a result of serological studies, accepts the general sequence of Ricciaceae (primitive), Marchantiaceae, Jungermanniaceae, *Sphagnum* (*Andreaea, Archidium*), Musci frondosi, *Anthoceros,* Pteridophyta (with *Psilotum* nearest to *Anthoceros*). The opposite view, that for the liverworts, radial organization of the gametophyte is primitive and the dorsiventral derived is supported by Verdoorn (466), Harris (202) and Eames (138). These authors give numerous references to earlier work by means of which the history of opinions can be traced. Fritsch (152) places the liverworts before the mosses and for the former has the sequence Anthocerotales, Marchantiales, Jungermanniales. A very full summary of the views on the phylogeny and classification of Hepaticae, from the publication of Synopsis Hepaticarum up to 1917, is provided by Schiffner (383).

The tracing of bryophytic origin or origins is highly speculative. Some gametophytic characters, *e.g.,* the chloroplast and pyrenoid in *Anthoceros,* suggest a more or less direct algal derivation. Some sporophytic characters, gross morphological and anatomical, suggest relationship with the Psilophytales. The group as a whole is markedly isolated from other plant groups in the combination of both gametophytic and sporophytic characters, so far as all known extant and extinct members are concerned.

Pteridophyta

In studying the phylogeny and its influence on the classification of vascular cryptogams, pteridologists have one great advantage over students of other groups of plants, except, perhaps, over those studying the Gymnospermae, in that a considerable body of relevant fossil evidence is now available for their use. Further, much of this evidence is summed up with numerous bibliographical references in a series of modern well written text-books (*e.g.,* 55, 58, 79, 467, 506, 138). A summary of earlier views up to 1908 is given by Browne (69). In spite of this advantage, the phylogenist is faced, even in the Pteridophyta, with a number of major difficulties. The fossil record is still very incomplete, the same facts are given very different interpretations by different specialists—

above all, as regards certain supposed organogenetic lines—and convergence appears to be rife. Probably it is in the Filicales that the nearest approach to a phylogenetic system has yet been made for any plant group. Bower, for example, says (55) : "The Ferns are much richer in genera, species, and individuals than any other living Pteridophytes. They probably present the climax of successful development in Homosporous Vascular Plants. They show also a high degree of variety both in their vegetative and in their propagative characters. These characters provide good diagnostic features upon which their Classification may be based. They have a full and long palaeontological history, which may be traced through successive horizons backwards to Palaeozoic times. The characters of the fossils have been proved to be so far comparable with those of certain of the living Ferns that their relationship cannot be held in doubt. The geological record can therefore be used as a valid check upon such conclusions as to relative antiquity as may be drawn from the comparison of living types of Ferns. There is in fact no group of living plants which offers so favourable an opportunity for phyletic classification; for none is there so long, rich, and consecutive a geological record, combined with so great a variety of living types, possessed of diagnostic characters so marked, and with progressions so susceptible of reasonable physiological interpretation. This gives the Filicales an interest peculiarly their own. But the classification of such a group need not have as its end merely the reduction of the group itself to phyletic order. It may be made a means of comparison applicable to other groups". Bower uses the word natural as synonymous with phyletic in reference to classification. Again and again he emphasizes the importance of palaeobotanical evidence, and yet, partly because of the "plurality of converging lines", he finds that "a natural, or phyletic, Classification is becoming increasingly difficult, and is of necessity complex. For it is not always sufficient to observe the physical characters presented; we also require to have some reasonable view or knowledge of how the characters of the individual observed were arrived at in its Descent". Again, "A complete artificial classification is always possible, and is indeed necessary for floristic use. A complete phyletic classification will only become possible with complete knowledge of the descent of the organisms classified. The second cannot replace the first under

present conditions, owing to the imperfections of present knowledge. But it can lead to a correction and amendment of classification for floristic use, so as to make it run ever more nearly along the lines of probable evolution". Bower gives a valuable analysis of the criteria of comparison used in obtaining his classification. An excellent concise summary of his conclusions is given (509). Stelar morphology, particularly of the ferns, is very fully considered, with numerous references, by Posthumus (341).

It has been widely held that the existing vascular cryptograms other than the Filicales represent more or less degenerate remnants of groups formerly showing much greater diversity in structure, having more dominant life-forms, and in general a much more important relative position in the world's flora and vegetation, particularly in Palaeozoic times. While this remains probably true in part, there have been various suggestions published which modify the view that existing forms are degenerate and suggesting that they may well represent persistent herbaceous series derived from unknown herbaceous ancestors and not from woody ancestors by loss of the power of producing secondary thickening, *etc.* That, for example, the ancestors of *Selaginella* and *Lycopodium* are not to be sought in the *Lepidodendron-Sigillaria* or similar series, or those of *Equisetum* in the Calamitaceae or similar groups, but in primitively herbaceous ancestors (*cp.* 124 and the literature cited there).

Considerable importance is given to the Psilophytales in all recent discussions on the origin of vascular cryptogams and also of the Bryophyta (21, 58, 70, 124, 223, 467, 506). The group certainly has synthetic characters, and further discoveries of fossil-bearing beds of earlier and middle Palaeozoic age may well lead to settling some of the outstanding questions regarding the basic connections between the main groups of cryptogams. While hypothetical trees continue to be published, many authors are inclined to "consider the major groups as forming a bundle of sticks".

A summary classification of the Pteridophyta is given by Fritsch (153) with references to literature.

Gymnospermae

In this group, as in the Pteridophyta, there is very much fossil evidence which is relevant to phylogeny. As in the vascular

cryptogams, vegetative anatomy is also of considerable importance. For the extinct groups, such as the Cordaitales, Cycadofilicales (Pteridospermae) and Bennettitales, possible origins from pteridophytic ancestors have been suggested. There seems no doubt that the Gymnospermae were in existence long before the Angiospermae, and not only must the establishment of the seed habit be traced, or mainly traced, through them but there is also the possibility that the ancestors of the modern Angiospermae are to be sought in one or more of the known groups of Gymnospermae. The best known of the theories linking the Gymnospermae with the Angiospermae is that maintained by Wieland in a number of publications and elaborated by Arber and Parkin (22). On this view, an ancestry for the Angiospermae is traced through a number of hypothetical forms (missing links) to the Mesozoic Cycadophytes, particularly to the Williamsonian-Bennettitean group. The great difficulty in this theory is in correlating the gynoecium of the cycadeoids with that of any existing or known fossil angiosperm. "There are no recognizable carpels in the Cycadeoids and no interseminal scales in the Angiosperms" (386). Since the Arber and Parkin hypothesis has been approximately accepted by Hutchinson (229, 230) for the basis of his classification, the following conclusion by Scott (386), who concisely and very clearly summarizes the evidence, may be given: "But we are still a very long way from tracing the ancestry of the Angiosperms. That there are striking analogies between the great modern sub-kingdom and the once dominant Cycadeoids of the Mesozoic is undeniable. It is also true that the analogies become accentuated if we take into consideration the older and more generalized Williamsonians rather than the later and specialized Bennettiteans. On the Angiospermous side, it is with such families as the Magnoliaceae and other Ranales that analogies can best be traced. But, after all, a wide gap remains. We cannot be certain that there is anything more than a parallel development; even so, the fact that the flower, using the word in its natural sense, was already a feature of the leading Mesozoic race, is in itself of great interest.

But it may be that a real affinity exists; that the Cycadeoids and the Angiosperms are branches of a common stock, and that the former deserve the name of Pro-Angiosperms which Saporta, perhaps with prophetic insight, long ago gave them".

The pros and cons of a possible phylogenetic connection between the Gnetales and other groups, including the angiosperms, are adequately summarized by Pearson (335). More recently Hagerup (185) has reached the conclusion that the Gnetales form a connecting link between certain gymnosperm and certain angiosperm groups. Hagerup (184), largely as a result of morphological (including anatomical), ontogenetic and teratological research, concludes that the coniferous cone is an inflorescence and the integument a macrosporophyll. As a result of these studies he traces a connection between the Coniferales and the Lycopodiales, not between the former and the ferns and cycads. Hagerup's conclusions are in the main supported, and even extended, with modifications for *Juniperus* and *Taxus,* by Laufer (264).

The phylogeny and evolutionary tendencies of the Gymnospermae themselves are discussed at length by Chamberlain (84, 85), by Coulter and Chamberlain (113), and by Campbell (79). Darrah (124) and Zimmermann (506) should also be consulted. All of these works contain many bibliographical references. One may note that, as in other groups, there are considerable differences of opinion on the subject and many authors believe that the major groups of gymnosperms have largely had an independent evolution and can not be reasonably linked up with other groups.

The pteridosperms, separated by Seward (390) from the Gymnospermae under the name Pteridospermophyta, are again coming into prominence as the possible ancestral group not merely of seedplants in general but even more or less directly of the Angiospermae. References to literature concerning them can be traced through Scott (385), Seward (388, 390), Zimmermann (506) and Darrah (124) in addition to the special books on Gymnospermae already quoted in this section.

Angiospermae

There is a very considerable but scattered literature on the origin and phylogeny of the angiosperms, commensurate with the diversity of opinion on the subject. A useful summary of the meagre relevant palaeobotanical evidence is given by Darrah (124). Most often the hypotheses, so far as they are based on what is considered the most primitive existing order or orders of flowering plants, fall into two broad groups: those accepting the Ranalean-Magnolian plexus as the most primitive and those looking on one or more of

the orders (Pandanales, Verticillatae, Salicales, Fagales, *etc.*) with simple flowers, albeit often with complex inflorescences, as the most primitive. With various modifications, and sometimes taking up a less extreme position, Wieland (489), Hallier (194), Bessey (44), Calestani (73), Hutchinson (230) and Copeland (110) expound the former, Engler (140, 141), Wettstein (485), Rendle (357), Hagerup (185) and Pulle (344) the latter, either in discussion of their principles and conclusions or in their systems. At present, palaeobotanical evidence gives little support to either view as opposed to the alternative, and it is probably true that the immediately ancestral forms of existing angiosperms are not yet known or not yet recognized. Burtt Davy (127) apparently accepts both the Querciflorae (Amentiferae, Fagales) group and the Magnoliiflorae (Magnoliales) group as primitive, but says that except perhaps in the Amentiferae unisexuality is advanced relative to bisexuality.

Anatomical research (176, 298, 286), pollen investigations (394, 395) and published sero-diagnostic work (247, 248) support the view that the Magnoliales are at least a very ancient and, in some characters, a relatively primitive order. Some at least of this evidence appears to be independent of the conclusions reached by comparative gross morphological studies and may thus break the vicious circle of primitive group—primitive characters—primitive group. The study of chromosome numbers (486) suggests that the Magnoliales are a peculiar group within the angiosperms and that their classification needs revision. On the other hand, the palaeobotanical evidence available shows that other orders, of quite different affinity, also occur as fossils in Cretaceous and Jurassic rocks and that these include some with simple unisexual flowers. There seem to be three possibilities: *a*) that the angiosperms are monophyletic but have had a very much longer history than is at present known, perhaps stretching back into Palaeozoic times and with a whole series of missing links; *b*) that the angiosperms are monophyletic but that the first and at present unknown group diverged quickly, in terms of geological time, into a considerable number of different groups; *c*) that the angiosperms are polyphyletic. These possibilities are not entirely exclusive one of another. The last possibility has been little considered in the detail it would seem to deserve. Campbell (76) remarks that "Both

comparative morphology and the geological record indicate that the existing Angiosperms represent a number of distinct phyla which cannot be traced back to a single ancestral type". Thomas (429) concludes that "The evolutionary tendencies already detected in these three groups [Caytoniales, Bennettitales, Pteridosperms] furnish reasonable grounds for the idea that the Angiosperms were derived from various Pteridosperms early in the Mesozoic period". On the other hand, Parkin (333) argues for the monophyletic origin of the angiosperms on the grounds of the stereotyped nature of the embryo-sac, that the same kind of stamen is found throughout the group, and that there is an invariable arrangement of the stamens and carpels in all hermaphrodite flowers. It is, however, doubtful that either carpel or stamen is so uniform morphologically or morphogenetically as Parkin believes, and numerous recent researches have shown that embryo-sac structure in the angiosperms and changes occurring in it both before and after fertilization are much less uniform than was at one time supposed (see review by Maheshwari, 284). Some of the most important arguments for the monophyletism of the angiosperms have, indeed, been decidedly weakened by several lines of recent research. A very careful comparison of perianth structure and ontogeny might well show that the perianth was not strictly homologous, even as calyx and corolla, throughout the angiosperms. Diversity in the structure of the gynoecium and of the androecium is also known to be very great, and the series based on gross external morphology may well be very superficial and fail to hold for other criteria. Researches of an organogenetic nature in the angiosperms are not at present leading to uniform interpretations, but when they can be combined with extended palaeobotanical investigations on fossil angiosperms and other Spermatophyta, they may well throw very considerable light on the origin of the group and help to establish lines of development. The history of the angiosperms is still almost as great a mystery as it was in the time of Darwin. What is most needed is further investigations of Jurassic and Permo-Triassic plant-bearing strata. The discovery of the angiospermous, or more or less angiospermous, Caytoniales suggests strongly that quite unexpected results may accrue from such investigations. We do not know when, where or from what the presumably most recent and now dominant large group of existing terrestrial plants arose. On

a priori grounds one might suppose that there would be more satisfactory knowledge of the phylogeny of the angiosperms than for any other group of plants. The absence of this raises the question as to whether some important method of evolution has been overlooked, still remains undiscovered, or has been given too little consideration. It may be that there is more truth than most botanists seem to accord to Lotsy's view (273, 274) that hybridization is the key to evolution. Anderson (4) has suggested on cytological grounds that the angiosperms may have arisen as a result of hybridization between two gymnosperms. If this be the explanation of their origin, the traching of their ancestry may be even more difficult than it would be if their origin and evolution were by accumulation of relatively small mutations. Hybridization and polyploidy are both commoner in plants than in animals, so far as our present knowledge allows of such a generalization, and though many basic details of sexual reproduction and the usual, or best known, mechanism of inheritance (Mendelian) appear to be essentially the same in both kingdoms, there are a number of important differences in structure and behavior which suggest differences in evolutionary mechanisms or in their relative importance in the two groups. If phylogeny be more difficult to determine in plants than in animals, one reason may be that there have been different methods involved in the evolution of animals and plants, or, at least, that some methods are more important in the one kingdom and others in the other kingdom, and that for plants the extreme of differences from animals appears in angiosperm evolution.

Those who utilize phylogenetic conclusions in classification usually commence with what they regard as the most primitive group and end with the most advanced group, the intermediate groups being arranged as far as possible in such a sequence that they are placed after groups from which they are believed to have been derived and before groups to which they are thought to have given origin. This accounts for the wide divergence between such systems for the angiosperms as those of Wettstein (484) and Hutchinson (230). Engler's system does not claim to be phylogenetic in the complete sense but to show in its sequence of groups progressive complexity of structure, apart from accepted subordinate reductions. Wettstein's system, in its starting point, depends largely on his view of the origin of hermaphrodite and perianthed from unisexual

and naked flowers. The latter type of flower is regarded as primitive in the angiosperms and as derived from a gymnosperm male inflorescence which develops by reduction into a male flower, and from a gymnosperm female flower, or much reduced female inflorescence. Hermaphroditism arose within the angiosperms from the male flower, in origin a reduced inflorescence, by the development of a female flower without a perianth at its center. Hutchinson's system accepts essentially the Arber and Parkin (22) view that the primitive angiosperm flower was hermaphrodite and provided with a perianth. Hutchinson then postulates a major division of the angiosperms into a group with "arborescent habit predominant" and a group with "herbaceous habit predominant". From the latter the monocotyledons diverged (in his first volume he says "in part", in his second volume his diagrams show as a whole). This heavy weighting of habit appears to be extremely artificial in the old sense of this term, in that, so far as it is maintained, it widely separates some groups having a very large number of characters in common, as the Cunoniales and Rosales separated so widely from the Saxifragales. It is also strange that in a system purporting to be based on probable phylogeny, certain groups are accepted as polyphyletic. The Asterales, on Hutchinson's scheme, are the most extremely polyphyletic of all possible groups within the angiosperms. It is true that there seems no *a priori* reason why in phylogenetic studies acknowledgedly polyphyletic groups should not be retained or made, but to do this would appear very much to detract from the special usefulness of phylogenetic schemes and classifications. One misses in Hutchinson's published accounts on the phylogeny of the angiosperms in relation to their classification, detailed explanations for the formation of major groups and of their arrangement. A critical review of Hutchinson's system has been published by Blake (50).

Hayata's dynamic system (204, 206, 207) is an attempt to explain and utilize the fact of widespread reticulation of characters within the angiosperms. Using Engler's system as a framework, he arranges around each family those families referable to the same series, together with those showing certain characters in common but not referable to the same series. In his fullest account (204) he gives reasons and numerous references. His explanations of the underlying causes of the dynamic system are less easy to

understand, perhaps because he wrote in, to him, foreign languages. It is hoped that the following much condensed account of his views is correct. The word gene is used by Hayata in a broader sense than in genetics. Different kinds of genes cooperate in the formation of a species and different species in different degrees participate in the same kinds of genes. Genes react differently in different combinations but are themselves essentially unchangeable except that they change from a latent to a potent condition and reversely. New genes do not appear and genes are not lost. This would appear to mean an original once-for-all appearance or creation of genes. New combinations of genes are brought about by crossing. Similarities of two individuals, of two species or of two other taxonomic groups, is due to their common participation of potent genes, their differences are due to the cooperation of different complements of potent genes. It follows that almost innumerable classifications can be made, according to the viewpoint of the systematist, *i.e.*, according to which characters he prefers. Every such classification is legitimate, and hence the only natural system must be a dynamic one, changing with the viewpoint of the systematist. Hayata gives numerous examples, mainly at the family level, amply proving the reticulate nature of affinities in the angiosperms. An entirely new and particularly interesting series has also recently been given by Hochreutiner (222), and very many more examples at all levels of the hierarchy of systematic groups could be quoted. Whether Hayata's cooperation-participation theory fully explains the facts is open to doubt. Du Rietz (135) gives a useful summary and criticism of Hayata's system and its principles. It is, perhaps, to the further development of cytogenetics that one must look for satisfactory causal explanations. Meanwhile, systematists might well attempt a tabulation of reticulations instead of merely listing examples of parallelism and convergence, as if they were unusual and isolated phenomena. This should be done at various taxonomic levels—species, genus, family, *etc.*—and for as many groups as possible. The results for all groups and levels should then be critically compared to ascertain if any general laws can be adduced.

Hagerup (184, 185) traces the origin of some angiosperms from the Coniferales through the Gnetales. His research, largely based on ontogeny, shows very clearly, as does so much recent research

work, the need for a complete overhaul of morphological categories and the homologies of organs, particularly those of the flowers of angiosperms. It also suggests that the possibility of a polyphyletic origin of the angiosperms should be carefully reinvestigated.

In recent times the importance of habit—arboreal or herbaceous—has assumed some importance in discussions on the classification and phylogeny of angiosperms. Eames (137), Sinnott and Bailey (398) and Sinnott (397), whose views are accepted by Darrah (124), conclude that the more primitive angiosperms were arboreal and that the herbaceous habit is derived. Arber (18, 19) adversely criticizes the evidence for the arboreal ancestry of the herb and puts forward the suggestion that the tree habit is an expression of racial senile degeneration and even a final expression of a certain fundamental tendency in plant life—"the expression of the liability to the accumulation of inert organic matter". Bower's views (56, 57) on the relationship of size and structure in plants appear to support this general contention. Hutchinson (230) considers that "In certain groups, trees and shrubs are probably more primitive than herbs". In his "Diagram showing the probable course of evolution of the dicotyledons", he separates, as mentioned above, at the base a branch with "arboreal habit predominant" from one with "herbaceous habit predominant", but in the sequence of cohorts and families he does not adhere to this division. A detailed careful survey of the taxonomic significance of habit and other features of the vegetative body of the angiosperms is a desideratum. Classification and phylogenetic speculation have within this group hitherto been based primarily on flower, fruit and seed characters. The degree of correlation between these and characters of the vegetative parts requires much further investigation than it has yet received. The prominence given by Hutchinson to habit may serve to direct the attention of taxonomists to aspects of plants, the importance of which has been recognized by ecologists.

Bews (46) believes that for the angiosperms the moist-tropical tree type is the most primitive. Derivative types of trees and shrubs tend to show such features as a) a greater localization of the reproductive processes both in time and space, b) the production of softer types of wood, c) a general reduction in height though not necessarily in the diameter of their stems, d) an increase of branching, e) a decrease in size of their leaves, f) increased bud protection,

g) thicker bark, *i*) an increase of minor xerophytic features. Under moist favourable conditions, the effects of the biota are of paramount importance, resulting in the production of various derivative types of plant form, lianes, epiphytes and terrestrial hygrophilous suffrutices and herbs. The herbaceous form in general, with increasing climatic differentiation, probably arose frequently by reduction in sizes of woody plants, giving a series of trees, shrubs, undershrubs and herbs.

Monocotyledones

As for the angiosperms in general so for the monocotyledons, we find a great diversity of views regarding their phylogeny and classification. Engler and Wettstein place them before the dicotyledons, regarding them as, at least structurally, a less highly developed group. Hutchinson places them after the dicotyledons, regarding the Ranales as the group from which they arose. Schaffner (382), who definitely states that taxonomy must be phylogenetic, says: "When it comes to the direct question as to which of the two classes of Angiosperms represents the more primitive condition, the answer is that in some respects the Monocotyls are the more primitive and in some respects the Dicotyls. The Monocotyls have apparently in their more generalized forms preserved the primitive tree type with little branching ability, as in Palms, Dracenas, Yuccas, Fourcroyas, and Ravenalas. The tree Ferns and Cycads have this general form of plant body, and from this type the trees with crowns of small branches, like Ginkgoes, Araucarias, and Magnolias, have probably been derived. The Monocotyl embryo with its single terminal embryonic leaf (cotyledon) is nearer the primitive fern embryo than the Dicotyl type. The Dicotyl embryo evolved in the Cycads, lower Conifers, Gnetums, and Dicotyls. On the other hand, the ring of open vascular bundles of the Cycads, Conifers, and Dicotyls is apparently a direct descendant of the vascular system of the lower Eusporangiate Ferns". Hallier and Lotsy (272) accept a polyphyletic origin; Hutchinson is emphatic for a monophyletic origin and regards similarities to groups other than the Ranales as due to parallel development (better, convergence). It is clear from anatomical and ontogenetic researches that structural monocotyledonary can arise in various ways (114, 111, 23, 226, 214, 276, 213). The phylogeny and classification of monocotyledons has been dealt with by many authors. Hutchinson (231)

gives an outline sketch of the history of the classification in this group. The very great diversity of opinion suggests that the criteria employed and the methods of investigation used are unsatisfactory. Reference should be made to (16, 17, 30, 42, 73, 77, 112, 117, 154, 211, 212, 269, 322, 374, 375, 416, 460, 473).

LOGICAL AS OPPOSED TO PHYLOGENETIC CLASSIFICATION

We have attempted to review in outline the history of plant classification, the criteria used in phylogenetic and taxonomic studies, and the latest views on the relationship of phylogeny and taxonomy in the major groups of plants. The position reached can not be regarded as satisfactory. Not only do we find great diversity of opinion expressed by phylogenists for one and the same group, but hypothesis appears frequently far to outrun any degree of certainty as based on known facts. This is to be regretted, firstly, because phylogenetic interpretations are so often used in schools and colleges as the basis for teaching taxonomy, and, secondly, because guess-work phylogeny brings the whole subject into disrepute. Phylogeny is a blessed word—it brings great comfort to the teacher who has to instruct his students in taxonomy, the more so if he has little knowledge or interest in the subject. More fundamentally serious is the basic uncertainty of much phylogenetic speculation in most groups of plants as a basis for classification because of the absence of relevant palaeontological data. Speculation, hypothesis-making, or imagination occupies an important position in science in suggesting new lines of research and new possible generalizations. Taxonomy, however, owing to its technique (descriptive, nomenclature) and still more to its peculiar position relative to other branches of biology which are so dependent on it for any intelligent presentation of their results, has to be relatively conservative. Hastily and superficially constructed new-fangled classifications do not receive general acceptance and even valid suggestions involving important changes are weighed with care before incorporation in relatively permanent taxonomic schemes. One reason why the greatest taxonomists, particularly for the angiosperms, have not accepted phylogeny as the basis of their systems is undoubtedly that they do not feel sure that the phylogeny is known. A second reason is that very practical classifications can be made without reference to phylogenetic speculation at all. A

third reason is that all the evidence derived from palaeontology, genetics, cytology and taxonomy itself points to phylogeny as having been so complicated that its mere description or expression in the form of diagrams is impossible without such simplification as largely detracts from the truth of the result. Complicated reticulations in three or more dimensions or on different planes can scarcely form the basis for an acceptable taxonomy.

Estimations of nearness or remoteness of phylogenetic relationship may be based on any one of the following:

a) The number of generations of the groups under consideration from a common ancestral group of the same taxonomic status.

b) The number or length of time periods, from years to geological eras, during which the groups under consideration have developed independently from a common ancestral group of the same taxonomic status.

c) The number of determined genom differences between the groups.

d) The number of considered phenotypic differences between the groups.

e) The summation of values assigned to abstracted phenotypic differences between the groups.

Most often it is not stated which basis is used; sometimes it is possible to tell from the data presented or the method of presentation; often it is obvious that there has been confusion of bases or the deliberate use of more than one.

One of the clearest expressions of the view that a natural classification coincides with a phylogenetic classification is that of Dendy (130), and the following quotations may well be taken as typical of the views held by many zoologists and botanists: "The aim which the zoologist or botanist sets before himself in classification is the expression of the natural affinities of the plants or animals investigated. The existence of such natural affinities was clearly recognized long before the explanation of them was known, and even by upholders of the doctrine of special creation. . . . It also soon came to be recognized that natural affinities could be best determined by taking into account as many characters as possible instead of relying merely on one or two. . . . the tree-like form assumed by a natural classification bears an unmistakable resemblance to the tree-like development of the whole organic world which evolu-

tionists believe to have taken place. The two results represent, indeed, but slightly different aspects of the same truth; the resemblance between them is no mere coincidence, but the fact that we are able to classify organisms in a tree-like manner indicates very clearly that these organisms have been produced by tree-like evolution.

The systematist is now able to replace the vague groping after natural affinities by a much clearer conception of the aims of taxonomy. What he has to strive after is the elucidation of the actual pedigrees of existing organisms, the unravelling of what is termed their phylogeny or ancestral history. This means neither more nor less than the ultimate reconstruction of the whole vast tree of life.

The parallelism between the two [the taxonomic tree of a natural system of classification and the phylogenetic tree] is sufficiently striking to justify us in believing that it would be complete if only our knowledge of classification and phylogeny were so; we should then doubtless see at once that the taxonomic tree and the phylogenetic tree are, after all, one and the same thing, for we should arrange all organisms strictly in accordance with the course of their evolution".

The last sentence makes very large assumptions, some of which are discussed elsewhere in this paper. Indeed, Dendy himself, after a discussion of cytological and other data, makes one important modification when he says: "In view of these facts, it seems desirable to modify our usual conception of organic evolution as simply tree-like, and to conceive of each separate branch of the phylogenetic tree as being composed of a very complicated network, due to the repeated union of male and female lines of descent. The same result would be expressed in human pedigrees if the female as well as the male line of descent were taken into account". If, indeed, evolution be sometimes divergent, sometimes convergent, sometimes parallel, sometimes linear, sometimes reticulate, sometimes progressive, sometimes regressive, the taxonomist may well think that the complications are too great for phylogeny to form the basis of any system of classification which shall also fulfil essential practical purposes.

Hall (188) quite definitely considered phylogeny as the basis of classification. Unfortunately, it is not clear that by phylogeny he meant more than an interpretation of the results obtained by classi-

fication on the basis of resemblances and differences and their correlation.

Durand (134) recognizes a number of classifications which are basically and logically different. He says: "La classification généalogique est la seule 'naturelle'; les classifications par ordre de ressemblance, autrement dit de généralité, sont toutes 'artificielles', c'est-à-dire basées sur certains caractères arbitrairement choisis. La classification rêvée, qui porterait sur la totalité des caractéres, m'apparaît comme une vraie chimère". His criticisms of Haeckel's attempt and, according to Durand, failure to superimpose a phylogenetic classification on a classification based on resemblance and his discussion of the phylogeny and classification of languages are worth the consideration of biologists.

Bower (53) clearly summarizes the sources of some of the weaknesses "under which views as to descent are liable to suffer": few authors realize how largely assumption figures in their arguments; they neglect collateral checks; they neglect the fact that intermediate steps between the organisms compared are not known; they use single characters for purposes of comparison. He shows that the Filicales answer requirements for phylogenetic study better than any other group of plants and believes that determination of their phyletic sequences may be scientifically conducted with high hopes of success. "If it be pursued in a broad scientific spirit, the result will probably serve as an object lesson, which may be applied in laying down the canons of comparison for other and still more difficult series of plants".

Meyer (301) distinguishes between typological and phylogenetic systematics. The former is the natural classification of organisms and in current taxonomy has become inextrinsically mixed with phylogenesis. It is highly desirable that the resulting conglomerate should be resolved into its different constituents. The system of metamorphoses gives the natural system, the summation of genealogical series (Phylogenesen) results in the phyletic system. "*Typologie konstatiert rein ideale Bauplanverwandschaft, während die Phylogenie als völlig neues, typologisch gänzlich kontingentes Moment den historischen Gedanken in die Biologie hineinbringt. . . . Genealogue organismische Systeme sind auch homolog, aber nicht alle homologen Systeme sind eo ipso auch genealog.* Eine weitere Prüfung dieses Sachverhaltes ergibt, dass Phylogenie über-

haupt garnicht auf ein taxonomisches System der Organismen tendiert. Sie hat als rein historische Wissenschaft ganz andere Aufgaben, die weit über alle blosse Systematik hinausgehen. Diese Aufgaben sind dann genauer zu schildern. Typologie ist Voraussetzung, aber niemals Ergebnis der Phylogenie".

Sprague (236) says: "taxonomy may be defined as scientific classification of the different kinds of living organisms according to their proved or inferred phylogenetic relationships". All the examples he gives, hypothetical and actual and apart from those he adversely criticizes, are, however, of classification on the basis of maximum correlation of characters, the results being interpreted as phylogenetic. This is the converse of classifying according to phylogenetic relationship. That classification supports or suggests a possible phylogeny is one thing, that classification is made according to phylogeny, proved or inferred, is another and one for which Sprague gives no examples that he accepts.

As early as 1874, T. H. Huxley (239) criticized adversely the incorporation of phylogenetic speculation in taxonomy. His own words are: "Valuable and important as phylogenetic speculations are, as guides to, and suggestions of, investigation, they are pure hypotheses incapable of any objective test; and there is no little danger of introducing confusion into science by mixing up such hypotheses with Taxonomy, which should be a precise and logical arrangement of verifiable facts". Rabel (345) quotes Kant, unfortunately without giving exact references, as distinguishing between classifications based on relationship and resemblance, respectively.

Caruel (80) clearly pointed out that a natural classification is one based on as many characters as possible. While all classification is artificial in being produced by an artifice of the human mind, classes based on a high degree of correlation of characters are more natural than those based on one or a few characters, since the degree of correlation can be determined objectively as it occurs in nature. The more characters that are correlated the more natural is the classification. Thus, the Cruciferae or Umbelliferae are said to be more natural groups than the Ranunculaceae or Rosaceae. Correspondingly lower grade taxonomic groups must be more natural than higher ones: species are more natural than families.

Gilmour (236) has dealt very clearly with some of the problems

of natural versus artificial classification. He shows that "a natural classification should rather be regarded, first and foremost, as that arrangement of living things which enables the greatest number of inductive statements to be made regarding its constituent groups, and which is therefore the most generally useful classification for the investigation of living things. Whether or not such a classification does in fact group together individuals who are phylogenetically related is a secondary question which must be answered for any particular case on its merits". Basically, "all classes of living things, taxonomic and non-taxonomic, though differing in their biological importance, should be regarded as of the same philosophic character, namely as rational concepts constructed by the classifier to clip together certain sense-data experienced by him". There is no difference, other than quantitative, between a natural and an artificial classification—"a natural classification of living things is one which groups together individuals having a large number of attributes in common, whereas an artificial classification is composed of groups having only a small number of common attributes"; the former "can be used for a wide range of purposes", while the latter "is useful only for the limited purpose for which it was constructed"; yet "both types are created by the classifier for the purpose of making inductive generalizations regarding living things".

Richards (359) emphasizes the importance of studying simple correlation between habit and structure, in spite of the great amount of convergence in the former. He suggests that the ultimate aim of [zoological] taxonomy is twofold: "(1) To arrange living animals in a hierarchy of groups which have an increasingly large number of characters in common. This can be regarded as a rough outline of the probable phylogeny of the group, though no such speculation is actually necessary during the performance of, at any rate, most of the work. (2) To make *predictions* from structure to function and *vice versa*". The second aim constitutes much of what may be termed practical taxonomy, and such predictions are widely made by taxonomists, often without a very clear realization of what is involved.

In discussing the meaning of phylogenetic relationship, Gilmour (236) points out that the phylogenetic taxonomist working with living groups conceives phylogeny as concerned with the origin of

groups. This group concept can not be independent of correlation of attributes because we must make our groups (phyla) before constructing our scheme of their origin and development (genesis). On the other hand, the lineage concept is based on actual genealogical relationship—or what is accepted as such—and is independent of correlation of characters (163). Since lineages can be traced with any degree of validity only when large series of fossils of the group concerned are available, and since, as we have seen above, the tendency is to regard lineages as themselves of very considerable complexity, it would appear that maximum correlation of characters must form the basis of the most natural possible classification of the vast majority of plant groups. Phylogenetic theory may well help to interpret the results of such classification, and in that sense phylogeny may "be regarded as forming a sort of background to a natural classification". Crow (115) argues very strongly that phylogeny is not genealogy. The present writer agrees, with the proviso that genealogical, lineage and gene studies, where they are possible, may throw much light on phylogeny. Genealogy is the tracing of the ancestry of individuals, phylogeny is the tracing of the origin and development of groups. An individual is genically constituted at fertilization, apart from somatic mutations, the gene content of a group may have had a long and varied process of origin and may be continually changing up to the extent of forming a new group or groups, *i.e.,* what have for scientific convenience to be considered as such.

Boyden (62) very clearly expresses conclusions in general agreement with those of Gilmour. Thus, he says: "We mean by the term animal relationship an expression of the degree of similarity in the essential natures of the organisms compared. In so far as the characters measured are determined by heredity, animal relationship coincides with genetic relationship. . . . In biology the real goal of taxonomy is to group organisms in proportion to their degrees of resemblances in essential hereditary traits. It has often been stated that the goal of taxonomy is the expression of phylogenetic relationships. Actually, the phylogeny is always inferred from the degree of similarity in characteristics believed to have genetic basis, and a classification is not based on phylogeny. Rather, both classification and phylogeny are developed from data regarding the essential, *i.e.,* hereditary natures of the organisms classified".

A valuable and clear criticism of so-called phylogenetic systems is given by Souèges (408). He points out their very great dissimilitude and says that the arguments produced in support of the supposed phylogenetic relationships are most often weak, incomplete and disputable. The strongest argument that can be levelled against them and the fundamental reason of their discordance and lack of success is that they are based upon purely static morphology. This results in judgments that are too subjective, too much matters of personal idiosyncracy. Truly natural classifications remain an ideal, yet one that should be kept in mind.

Anderson (10) concludes that "it would be well if monographers could approach their work with minds unprejudiced by evolutionary theories. We are so certain of the fact of evolution that we are prone to forget how little we know about the forces behind it". The transforming of confusion into order is the great satisfaction of taxonomy.

Bremekamp (65) urges that taxonomists should abandon efforts to construct phylogenetic trees, which he apparently regards as impossible of success, and should classify organisms in a natural, *i.e.*, logical, system, from which subjective judgements of sequence are excluded. In a later article (65a) he shows the essential untrustworthiness of so-called phylogenetic systems by critical consideration of their own principles. He says: "We come therefore to the conclusion that the arrangement of the units of a group in an ascending series is impossible; a direct determination of their age is out of the question, because the historical evidence is entirely inadequate, and the methods for an indirect determination are untrustworthy. Order should be created in another way, namely, by increasing the number of subdivisions; in this way the number of units per group decreases, and they are more easily surveyed".

The writer feels he must here interpolate a few personal views. Such words as natural, artificial and real are used in discussions on taxonomy and phylogeny with such a wide diversity of connotation or so ambiguously that it might be well entirely to replace them in such connections by new terms, carefully defined, such as general and special, general classifications being based on correlation between the largest possible number of characters and special classifications being based on correlation between a limited number of deliberately abstracted characters (*cp*. 163a). The very great value

of special classifications needs emphasis. Thus, a classification
whose aim is ease of determination of specimens is a special classi-
fication whose success is capable of pragmatic evaluation. Special
ecological, Raunkiaer's (351), sterility-fertility, as Danser's (120),
or cytological classifications have value not only in throwing light
on special problems but in providing, analyzing and synthesizing
data which must be considered in the building up of a general
classification. Thus, modern taxonomy is given a much more
varied, a much wider, and a much more interesting aim than that
of merely trying to construct phylogenetic trees. Its aim is to in-
corporate all biological knowledge in classification. This must in-
volve much experimenting with new methods and new technique,
for many of the data from modern research in phytogeography,
ecology, genetics and cytology differ much from the morphological
data on which the classical systems are entirely or mainly founded.

These views have been, in essence, recently expressed by Gilmour
and Turrill (163a). They point out not only the value of special
classifications but that the relationship of such special classifications
to general classification is not always as clear as might be desired.
Special classifications "exist in their own right for special purposes".
While they are based on a limited number of abstracted criteria,
classifications which must be regarded as *stages* towards a general
classification may also, indeed owing to the imperfections of knowl-
edge and the limitations of methodology must, temporarily be based
on an abstracted number of attributes. It is only by a clear defini-
tion of purposes for which a given classification is proposed that
confusion between special and incompletely general classifications
can be avoided. The special classification is, in theory, complete,
but static stages towards a general classification are only temporary
halting places in an otherwise dynamic advance.

PHYLOGENETIC DIAGRAMS

The classic form of a diagram to express supposed phylogenetic
relationships is that of a tree. Diels (131) gives examples of meth-
ods commonly used in attempts to symbolize and diagrammatize
phylogenetic schemes, and the historical aspect of these attempts is
traced in some detail by Lam (253). Haeckel's notion of a tree
trunk with branches has been much crticized and modified by sub-
sequent writers. Modifications sometimes take the form of a shrub,

of a liane or a bundle of sticks; sometimes they are conventionalized as into a number of linked circles. Typical examples of branched diagrams to illustrate phylogeny are provided by Macfarlane (279, 281). A common feature of such tree or modified tree diagrams is the absence of roots, suggesting that no very strong force is needed to overthrow them. Some diagrams express at most only degrees of (generally gross morphological) differentiation. In others, by various devices, attempts are made to indicate the geological time factor. In still others, the factor of geographical range is introduced. The value of all such diagrams depends on three factors: *a*) the soundness of the research on which they are based; *b*) the ingenuity of the author in devising symbols and technique to express as many of his conclusions as possible in a concise diagrammatic form; *c*) recognition of the limitations of such diagrams and that at best they no more than represent their authors opinions.

Lam's (253) scheme of concentric circles and his three-dimensional fundamental scheme appear to be very considerable advances in methods of expressing phylogenetic conclusions. Handel-Mazzetti (196), for *Leontopodium,* also shows relationships of existing species in a three-dimensional conventional manner. It is, however, becoming more and more clear that evolution has been extremely complicated in the sense that it has occurred by a great variety of mechanisms (gene-mutations, chromosome mutations and aberrations, genom-mutations, hybridization, with the innumerable sieves of natural selection, *etc.*) and at very different rates in different groups and at different times and places. Any attempt to indicate phylogeny in diagrams must mean extreme simplification and the risk of the well known dangers of over-abstraction. A further series of errors may result from the mental construction of hypothetical ancestors and hypothetical missing links. In so far as diagrams are used to express degree of resemblance and difference, *i.e.,* of taxonomic relationship, they can be pragmatically tested and may be extremely useful. Actually, as Lam (254) has pointed out, taxonomic schemes are cross-sections through phylogenetic schemes, assuming that the taxonomic scheme does express, as far as is possible by this method, the relationships of the units which are considered as static. Taxonomy is based on characters, phylogeny on changes of characters. The units of phylogeny are

"streams of potentialities", for any one of which Lam proposes the term genorheithrum. The aim of phylogenetic diagrams must be to show the genorheithric connections between the more or less disconnected taxonomic units. A criticism of some of Lam's views has been published by Shaparenko (391).

In an earlier part of this paper reference was made to pre-Darwinian authors who considered that a net-work was the best analogy of plant affinities. This view is supported more and more by recent researches. Anderson (3), for example, referring particularly to the Liliiflorae, says: "Phylogenetic relationship and morphological resemblances in such groups will be reticular rather than dendritic. That is to say the course of evolution, instead of being represented as a tree with diverging branches, may be more aptly likened to a complex and irregular web, with threads of varying thickness". Hayata (205) stresses the importance of hybridization in evolution and believes that, at least at the species level, a net rather than a family tree must be used to represent genealogy.

CONCLUSIONS

"Botanical classification, when complete and correct, will be an epitome of our knowledge of plants" (Asa Gray, 169).

A great many different and sometimes radically opposed opinions and conclusions as to the actual or possible connections between taxonomy and phylogeny have been quoted or paraphrased in this essay-review. An attempt has been made to select as great a variety of opinions and conclusions as possible. Many have been missed out, partly because some appear to be too ambiguous to be of much practical use to anybody, but partly because of the difficulties under which this paper has been prepared. Collection and checking of references, and even concentration itself on a highly theoretical subject, are not easy under war conditions. Quotation or reference, with or without comment, does not necessarily mean that the writer agrees with the opinions or conclusions of an author.

Modern biologists accept the general thesis of evolution: that the wealth of animal and plant kinds now existing or whose relicts can be studied as fossils have originated from pre-existing kinds by processes which can be investigated, directly or indirectly, by scientific methods. It should be theoretically possible, therefore, given sufficient data, to trace the production and development of the kinds

of organisms as these are grouped by systematists, however complicated the results may be. The study of phylogeny is, indeed, not only justifiable as a deduction from the general theory of evolution, but in its own inherent right is a subject of great biological importance and interest. Historically, classification preceded, and must precede, investigations of phylogeny. The groups (phyla) must be determined before their origin and development (their genesis) can be investigated. The existence of groups showing a high degree of correlation of characters and of graded series of changes in such correlation are two of the most important proofs of evolution, not *vice versa*. In spite of this fact, which appears such a truism to the writer, there are still two schools of thought amongst biologists concerned with the relationship of taxonomy to phylogeny. The first states that phylogeny is the only basis for a truly scientific system of classification of plants and animals; the second accepts a broader basis for a general classification in attempting to utilize all available data and constructing the classes on the maximum correlation of characters, discontinuities and breaks in correlation determining the boundaries of the classes. This distinction into two schools is not absolute but, in general, can be traced through a great many of the quotations and summaries of conclusions given in this paper for a wide range of biologists. In many discussions dealing with taxonomy and phylogeny at scientific meetings, two such schools can be distinguished. This is easy, for example, in the discussion on "Was verstehen wir unter monophyletischer und polyphyletischer Abstammung," published in Verhl. Zool. Bot. Ges. Wien **59**: 243. 1909, and in the discussions held recently in private and public (163) under the auspices of the "Association for the Study of Systematics in Relation to General Biology" to which the present writer is greatly indebted for increasing his knowledge and breadth of outlook on the theoretical aspects of both taxonomy and phylogeny. Phylogeny apparently appeals strongly to many of those engaged in museum and herbarium studies—it breaks the monotony of dissecting, describing and naming dead remains. It also appeals to the teacher as an easy means of sugaring for his students the insipid pill of descriptive taxonomy. A good example of the ease with which phylogenetic speculations can be made and published is provided by Lamb (259), and the footnote on p. 266 of Lamb's paper illustrates the point

just made. Church's paper on systematy of angiosperms (96) is another striking example. There are thus quite definitely psychological reasons for the popularity of phylogenetic speculation as related to systematics. Usually, the palaeontologist, who alone has the material for a direct study of past phylogenies, the phytogeographer or ecologist who studies the end results of such phylogenies in their interactions with their environments, including one another, and the cytogeneticist who studies phylogenies in being or in the making though on a small scale, are cautious in their statements as to the probable course of phylogeny, or, alternatively, limit their conclusions to a relatively small group of organisms. So much of what is termed phylogeny rests on deductions from assumed premises which have not been subjected to adequate tests of their validity. It is, indeed, pseudophylogeny. A plea for the use of more inductive methods—especially in the angiosperms— seems opportune. Sprague (410) criticizes certain supposedly phylogenetic classifications on the ground that no reasons are given in support of the assumed phylogenetic relationships. Such classifications are, like *nomina nuda* in nomenclature, mere encumbrances.

If it be allowed that classification is a grouping into classes and that phylogenies can, with sufficient data, be determined, there is still a main problem of how far a known phylogeny can be incorporated in an improved classification. Classes of one hierarchical level, once formed, can be arranged on a system, and the systematist has been said "so to organise his pigeon-holes that their symmetry or subordination was an intellectual satisfaction in itself and a stimulus to further research" (339). How far this making of a system involves more than a further step in classification—a grouping into larger classes of smaller classes already formed—it is difficult to say, except that arrangements of units (or classes) within a class of any size does not appear to necessitate the making of intermediate size classes. It is in this arrangement that phylogeny may add to the usefulness of the system. In the actual checking and improvement of made classes the application of phylogeny, when known, may lead to a more generally useful system by reducing the number of polyphyletic classes, is so far as this is consistent with other aims of classification. By determination of an ancestral, or at least of a primitive, group, phylogenetic studies may give a starting point for a sequence which will, within limits,

help in the construction of a classification more widely utilitarian than it would otherwise be. For most practical purposes a linear classification is essential, as for monographs, floras, handbooks, museums, herbaria and botanic gardens. Probably no phylogenist would claim a linear sequence as phylogenetic except for very small classes or groups of classes. This gives an important limitation to the use of phylogeny in a general classification.

It is clear that phylogenies—whether studied for lineages or for taxonomic groups—are exceedingly complex. The detailed studies of lineages, mainly for invertebrate animals, cytological research proving innumerable chromosomal complexities, and genetical analysis all point more and more to this conclusion. Divergence, convergence, flow of genes, gene participation, hybridization, polyploidy, all these and no doubt many other phenomena have influenced or determined the course of phylogenies whose unravelling appears increasingly difficult, though always more interesting to the biologist. The result must, however, be that the more phylogeny is known the more is it found to be unsuitable as a *basis* for classification for general purposes. Bather (34) very pertinently remarks: "A nomenclature and a classification based on ascertainable fact will enable us to construct any number of phylogenies according to each worker's interpretation". Pledge (339) notes that "since parenthood is only one factor in life, there is no reason why an evolutionary classification should have been the most convenient for all purposes". A re-classification on the basis of phylogeny, in so far as it be possible, is a special classification, valid within its own limits and essential for its own purposes. The incorporation of phylogeny, so far as it is advisable and possible, in a general classification should apply only to reasonably proved phylogeny and not to mere phylogenetic hypotheses.

A very practical matter will have to be more fully considered by taxonomists than has so far been done. Taxonomists are more and more extending the list of characters they use in classification. How far is it possible to pour new wine into old bottles? There is, for example, an increasing difficulty in fitting the results of detailed modern studies in autecology and genetics into the existing scheme of taxonomic nomenclature. This is true also for phylogenetic research (see Lang, 261). If one tries to express ecological, genetical or phylogenetic relationships, one has frequently

either to adopt new methods of expression usually involving not merely a new terminology but a new or modified nomenclature, or to effect some more or less confusing compromise with existing taxonomy. The problems involved are probably solvable; they are, therefore, recommended to the urgent attention of biologists.

The task of the systematist is twofold: to prepare as many special classifications as are needed for special biological investigations, or to make available materials for others to construct such special classifications, and to make a general classification which shall express as far as possible in rational order all that is known concerning plants and animals. This last is an ideal which, even if never attained, is one which may well make the systematist proud in the magnitude of his task. It is an ideal greater than the phylogenetic ideal which is included in it and one which in the process of attempted attainment must make taxonomy what it should be, the focal point of biology.

EPILOGUE

The epilogue (Durand, 134) must remain in the original French; it could not have been written by an Anglo-Saxon and will not bear translation:

La *Taxinomie* est la science de l'ORDRE.

Et l' ORDRE, qu'est-il? Il est la condition suprême du bien, de même que la confusion, le trouble, le chaos sont source de tout mal.

Découvrir la vraie place de chaque chose, et l'y mettre si elle ne s'y trouve déjà. Voilà le souverain but de la science et de l'art, et c'est la TAXINOMIE qui en ouvre le chemin.

La confusion et le désordre, ce sont les ténèbres, c'est l'impuissance, c'est la stérilité, c'est la misère, c'est la souffrance; c'est la déperdition et le gaspillage des forces désorganisées se dépensant en frottements, contrecoups et entrechoquements douloureux.

L'ORDRE, c'est l'organisation normale, c'est l'organisation parfaite, où toutes les parties sont agencées suivant leurs vrais rapports de nature, et fonctionnent librement.

L'ORDRE, c'est la Liberté.

Il est la lumière, il est la force, il est l'harmonie, il est la beauté, il est le bonheur.

Honneur à LA SCIENCE DE L'ORDRE!

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XEROTHERMIC THEORY

PAUL B. SEARS

Oberlin College

I. INTRODUCTION

This theory assumes that there was at least one segment of time since the last major glaciation during which the climate was drier and warmer than at present. The problems raised by this supposition are numerous, difficult and often controversial. Two of them are paramount: (*a*) whether in fact such a period (or periods) existed and (*b*) if so, when.

Xerothermic theory had its origins in the attempt to explain the presence of living disjuncts, both species and communities, forming outliers in regions less continental than the main expanse of their normal present-day distribution. It has been reenforced by the finding of plant and animal remains in similarly anomalous locations. Its proof or disproof calls for evidence from a number of scientific disciplines.

The importance of the theory is considerable. In its broad outlines, our present fauna and flora is essentially Tertiary in origin. But the facts of present distribution in glaciated areas are to be explained largely by the events of Pleistocene and subsequent time. Thus the effects of a xerothermic period should be far from negligible. It is also important, for many reasons, to know the history and probable trend of climatic change since the last of the four great ice advances of the Pleistocene.

The Pleistocene represents the most recent of several prolonged periods of continental glaciation that have occurred in geological time. The three intervals between its four great glaciations have each been characterized by increasing warmth, a warm maximum, and gradually decreasing warmth. If the xerothermic theory is confirmed, there will be reason to suppose that we may be living in a fourth interglacial phase of the Pleistocene climatic rhythm, rather than in a truly postglacial period as we commonly assume. This would be true even though it might not be proved that the "postglacial" warm maximum was also a dry maximum.

The approach to any reconstruction of the past, particularly of its climate, must be cautious, tentative and open-minded. Here as

elsewhere in science, the maxim *"Causa non multiplicanda"* applies. The simplest hypothesis that will explain known facts is to be chosen. Hence the widely held view that with retreat of the last or Wisconsin continental glacier in North America, the climate gradually warmed until it reached its present character which it has retained with minor fluctuations ever since (13). The chief concern of those who hold this simple theory would be to trace, by fossils and living relicts, the course and rate of northward recession of arctic and boreal climates.

In opposition to this simple theory are various xeric, thermic or xerothermic theories, as will be seen.

It is important to note that glaciation affected a vast territory in both the Old and the New World. Retreat was marked by periods of rapid melting, long pauses and occasional readvances. Fluctuations in humidity which might be significant at one place might be masked in another. The same may be said for temperature changes. Conflicting views, based upon local differences, are to be expected.

II. ORIGINS OF XEROTHERMIC THEORY, 1800–1881

Plants and plant communities are often found outside the boundary of their principal ranges. Such a phenomenon calls for explanation. This is true whether the specimens in question are living or fossil. Northern vegetation, both living and preserved in organic sediments, is commonly found throughout the area once covered by Pleistocene glaciers. This is consistent with the idea that plant life moved south before and followed north after the glacial ice in the northern hemisphere.

The record is complicated, however, by the fact that traces of plant life characteristic of warmer, as well as drier, more continental climates are also found in glaciated regions. Such vegetation is properly designated "xerothermic" or "xerotherm" (Greek *xeros,* dry; *thermē,* warmth), and its presence in either the living or fossil state is regarded as evidence that the local climate was once drier or warmer, or both, than at present. This supposition, however modified in detail, is known as the xerothermic theory (29).

The writer has been unable to learn who first employed the term. This is not surprising, since its two Greek roots have long been in familiar use by scientific men, notably in medicine.

The theory has obvious importance for geology, climatology and

archeology, nor is it without interest to the layman. Its development was related intimately to the great scientific ideas of the nineteenth century. And for a generation—from about 1880 to 1910—it was the focus of bitter polemic. Throughout this dispute, and down to the present, the recurring influence of threads of thought, of geographical and technical backgrounds, and certainly of temperaments, makes an absorbing study for the historian of science.

The idea of plant migration was not at first self-evident. The burden of proof rested heavily upon anyone who advanced it as an explanation of plant distribution. By 1800 the accumulation of data had become so great that the Doctrine of Special Creation, in its fixed and rigid form, was no longer the fairly rational scientific hypothesis that it had once been. As early as 1747 Gmelin (30) had concluded that a given species might be created independently at various places. Yet the break with other ways of thinking was slow and difficult. Cuvier's theory of the cataclysmic destruction of old forms and creation of new is evidence of this (17).

The botanist Willdenow, however, tried to account for present distribution of plant life by assuming an original center of creation from which existing plants had variously migrated (80). His notion had strong logical roots in Linnaeus whose "Philosophia Botanica" Willdenow edited in its third edition. Here occur the following quotations, whose implication gives us, clearly enough, Willdenow's theory (45):

"Nova creatio nulla; sed continuata generatio".   (p. 39)

"Initio rerum, e omni specie viventium unicum sexus par creatum suisse contendimus.

".   .   .

"Disseminatio Naturae stupenda est.

".   .   . Erigeron 3. Hort. cliff. 407 ex America disseminata per Europam".   (p. 89)

On the other hand, Schouw (60), an early environmentalist, was conversant with the similarity of plants in similar though separated habitats. Neglecting the equally cogent observation of Linnaeus (45) that plants can and do spread, he pronounced migration an unnecessary assumption. In its place he revived and modified the suggestion made by Gmelin in 1747. He really made a double assumption, *viz.*, that plants are the product of environmental conditions, and that different regions could produce the same plant spe-

cies provided they had the same or similar conditions of climate. The effect of Schouw's doctrine of multiple origins was to throw the idea of migration into temporary eclipse. Agassiz (2), setting himself against the doctrine of organic evolution and indeed against the logical implications of his own theory of continental glaciation, remained an advocate of Special Creation. The influence of this doctrine is discernible even today.

In the face of such august opposition, the idea of migration was restored to favor by evidence contained in two important papers, one by Steenstrup in 1842, the other by Forbes in 1846.

Steenstrup (69) described the plant remains found preserved in successive layers of bog peat in Denmark. Beginning with the deepest layer, he found the following change of content: (a) *Populus tremula* with *Hypnum* and *Sphagnum*, (b) *Betula*, (c) *Pinus sylvestris*, (d) *Quercus sessiliflora* and *Q. pedunculata*, (e) *Fagus*, (f) existing moorland species. Here was indisputable evidence that during the gradual filling of a lake basin the kinds of local forest trees had changed. Since all the plants involved were stable enough members of the existing European flora, any resort to cataclysm or special creation to explain the change must seem less reasonable than a simple shift of distribution, *i.e.*, migration.

The doctrine of organic migration received even more substantial support from the work of Forbes (26). Although described by Darwin (18) as "strongly opposed to the views I maintain", Forbes did believe in common descent of the individuals of a species, and also had a strong sense of the kinship within a genus. He was well acquainted with fossils and marine organisms. He had at hand the excellent studies of Watson and others on the floristics of Great Britain. He followed Lyell's ideas in geology, recognizing the Glacial or Pleistocene, but shared the then common view that glacial drift was the work of a great arctic sea. He rejected Agassiz' idea that glaciation had produced a "cataclysm" and wiped out all life, but with it he discarded the sounder notion of continental ice-masses.

It was Forbes' merit to have adopted the geological notion of uniformitarianism for biology. He showed the reasonableness of assuming orderly movement of species in the absence of climatic and topographic barriers. He saw clearly that the flora and fauna of Great Britain and of the adjacent seas had various geographic

affinities. In his opinion the several elements represented had had their origin elsewhere and had entered at respectively suitable times. For this supposition he gave such evidence as he could in the form of geological history.

He mapped the present and former extent of three major regions: (a) the glacial-boreal, including the arctic, (b) the southern—Mediterranean-Atlantic, (c) the Germanic or central European. He showed both (a) and (c) as once in contact in the vicinity of the British Isles, but now separated by intrusion of the intermediate Germanic province.

Forbes established the presence, both living and fossil, of a cool northern biota, and a warm continental one in the intermediate temperate oceanic region. Thus he laid the groundwork for a structure of data which has steadily grown since his time, in both Europe and America.

At the same time he reported that the fossils of Mediterranean organisms were to be found in a layer (the Coralline Crag) below one containing boreal remains (the Red Crag). This led him to conclude that warm continental conditions had definitely preceded glaciation. He knew nothing of the evidence for repeated Pleistocene glaciations and prolonged interglacial periods.

The reasonableness of his view can not be questioned. There is today ample evidence of warmer conditions than the present, in both the Tertiary and at least one interglacial period. Making due allowances, Forbes' explanation is the prototype of the still current and vigorous belief that relicts or remains of xerothermic vegetation in glaciated regions are due to Tertiary conditions. Allied to this is the opinion that such relicts had an origin not later than interglacial, or at most late glacial.

On the other hand, Steenstrup's data contained the germ of another possible explanation. Denmark has today a humid oceanic climate. Yet the peat layers showed that between the cool boreal poplar-birch and the definitely humid beech there had been an interval of pine followed by one of oak. This sequence might indicate drying and warming ultimately followed by more moisture. In both Europe and America the beech is restricted to more humid areas, while the oak ranges into the interior continental climates. Thus Steenstrup's findings became a cornerstone for those who would attribute xerothermic relicts and remains to postglacial conditions.

Actually Steenstrup's evidence on this point is equivocal. In many places beech and oak coexist, with beech the normal ecological successor of oak. Vaupell (73) in 1857 presented a study of the invasion of beech into Denmark. He secured data from submerged forest beds, tufa, peat and historical sources, and was able to confirm the idea that beech was the most recent major forest genus to enter the peninsula. He held that it descended from the mountains of central Europe towards the Low Countries by way of the Baltic—a view beautifully confirmed by the "isopollen line" method of Szafer in 1936 (71).

Vaupell pictured western Europe covered by birch and pine at the beginning of the "present geological period". These were followed by oak, and the latter by beech. But since these genera all will grow in the same general climatic region, he brushed aside the fact that while their ranges overlap they do not coincide. He regarded any appeal to climatic change as unwarranted. He sought, rather, an edaphic explanation, in terms of "essences". The final entrance and establishment of beech he considered to have been aided by drainage and humus. As with the views of Forbes, much that Vaupell said cannot be questioned.

The defect of Vaupell's paper is that of special pleading. It seized upon Forbes for support of the idea of plant migration, but disregarded his evidence for climatic change. Yet it did marshall much evidence for edaphic succession, a subject to be neglected for a generation thereafter by serious scholars. Thus it established the existence of a factor that must always be taken into consideration in attempting to reconstruct the past. A strong emphasis on edaphic succession is naturally to be looked for in those who tend to simplify the course of postglacial climate. In this sense, Vaupell's influence still persists.

In 1855 appeared DeCandolle's treatise on plant geography (19), and in 1859 Darwin's "Origin of Species" (18). Both assumed the reality of migration and its control by climatic and other barriers. The key to present patterns was thus largely seen as lying in the past. In these views, now prevalent but then still hotly contested, Asa Gray concurred fully, as his published reviews prove.

In his Flora of Japan (1859) Gray (35) joined DeCandolle and Darwin against Schouw and Agassiz with their idea of multiple origins. He saw an explanation for discontinuity of distribution

in climatic and other changes.   Faced with the problem of some 134 species common to Japan and eastern north America—41 of them peculiarly so—and knowing the work of Heer and others on Tertiary floras, Gray used the ideas of DeCandolle and Darwin to fit together the pieces of his puzzle.   *The result was a definite theory of a postglacial thermic, if not xerothermic, climate.*

Briefly, Gray postulated a northern mingling of Tertiary temperate floras, followed by a Southern migration ahead of the advancing ice.   This in turn was followed by a northern return and contact again between the temperate floras of Occident and Orient.   Thereafter came a second southward retreat of these floras and transition to the present.   No interglacial periods are considered, and except for the fossil evidence of a circumpolar Tertiary flora, the evidence used seems to be mainly floristic.   There is no way of knowing whether Steenstrup's work was familiar to Gray.   The two were correspondents on taxonomic matters (56).   Gray was omnivorous and acute, and might have been expected to see the bearing of this evidence.   But he does not mention it.

In any event, Gray had not only dealt a vigorous blow to multiple origins and cataclysms; he had made it possible to think of postglacial time in terms other than a simple warming down to the present temperature level.

A decade later (1870–1) Kerner (40) produced what was, according to Gradmann (33), the first actual *xerothermic* theory of postglacial climate.   He invoked it to explain the isolated occurrence of southern plants in the eastern Alps.   Under the doctrine of migration, the only alternative would be to assume that these stations had not been covered by the glacier, nor sufficiently affected in climate to exterminate a previous warm-climate flora.

By this time the problem began to open up in other fields.   Loess, a fine silt-like powder forming great deposits, was frequently found where there was no evidence of aquatic sedimentation.   In 1878 von Richthofen (57), following the work of Kerner, announced his theory that loess was wind-borne dust associated with properly dry climate, during a portion of postglacial time.

Also Nehring (47), as a result of several years' study of mammalian remains, concluded that continental steppe-like conditions had followed the tundra and preceded the forest over much of western Europe.   The forest animals, bear, stag and squirrel, fol-

lowed the steppe animals such as the lemming and whistling hare. In addition, Nehring cited the presence of relicts of steppe flora; the need for a steppe climate to melt the great glaciers; the loess; and finally the better preservation of early animal remains than is usual in woodland.

There was nothing to suggest the old cataclysmic ideas in Nehring's work. He emphasized the fact that trees, coniferous in the north and deciduous in the south, are associated with the steppes of Russia and Siberia. The former steppes of western Europe, in his view, while continental, were not of the extremely dry type, nor was it necessary to assume a universal expanse of steppe. While definitely a phase of xerothermic theory, Nehring's emphasis is on relative dryness rather than heat. The reverse, it will be recalled, was true of Gray's (35) notion regarding early postglacial time.

By placing his steppe-period immediately after the tundra and before the forest, Nehring locates it very early in postglacial time. Thus it would apparently antedate the forest-bearing peat layers studied by Steenstrup. Here is another important source of divergent views, for Blytt (10) and others later leaned heavily on peat profiles for evidence of xerothermy.

The American equivalent of the steppes are the prairies and other grasslands. These were brought into the picture by Asa Gray in a review written in 1878 (36) dealing chiefly with the historical factor in vegetation. There was by that time a growing literature on the origin of American grasslands, most of it beside the point. Gray recognized the limiting character of moisture, and the generally greater aridity of grassland climate than of forest. He acknowledged the puzzle of the eastern prairies, outliers within the forest, concluding that there "must be a debatable border where comparatively slight causes will turn the scale either way".

Admitting the role of climatic change as a factor, he reiterated his belief that a milder postglacial climate than the present has prevailed in America, with vegetation moving northward in response and later retreating.

In 1875 Axel Blytt of Christiania (10), who had for some years been occupied with a study of the Norwegian flora, put forth the theory of the origin of this flora which is still associated with his name. His theory rested upon an hypothesis of postglacial climatic change which came ultimately to be known as the Blytt-Sernander

hypothesis, and a storm center of controversy. The account of Blytt's ideas most accessible to American readers was published and abstracted in 1882 (11).

Actually Blytt attempted to do what Forbes had done in 1842 for the flora of Great Britain. He distinguished six floristic groups in the generally uniform plant life of Norway. Each appertains to a different climatic center outside of Norway, and each is shown by his map to have a peculiar, somewhat disjunct range inside of that country. These groups he designated as *Arctic,* Subarctic, *Boreal,* Atlantic, *Subboreal,* and Subatlantic. Those in italics are continental (xeric). The other three are oceanic (humid). The order given is the order in which Blytt believed them to have entered Norway. It will be noted that this involves an alternation of dry and moist climates. In addition, the Boreal, the Atlantic and the Subboreal are considered to have been warm.

Blytt fixed the order of invasion by assuming that the coast of Norway had risen steadily since glaciation, and by comparing deposits, both of peat and of marine shells, at various levels above the sea. The order of his first four periods rests upon their temperature relations and the evidence of a gradual warming from glacial times to the Atlantic or 4th period. The Subboreal and Subatlantic are placed later than the Atlantic because their representative floras are confined to lower levels in Norway.

Following the suggestion of Geikie (28), who found evidence of a former rainy period in Scotland by studying peat deposits, Blytt investigated those of Norway. He reported a widespread alternation of peat with weathered forest remains. The peat he believed to indicate moist conditions, the forest drier periods. Where forest remains were lacking, he reported that sharp weathered boundaries might exist in the peat to mark dry periods. This is the forerunner of Weber's famous Grenz-Horizont in Germany (78).

Blytt reported, in agreement with Steenstrup's findings for Denmark, that Norwegian upland moors showed four layers of peat. These he said were separated by three forest layers, with a forest layer forming on top, due to recent drying. In addition he reported a clay layer with arctic tundra plants below, as found by Nathorst (46) in 1870, and traces of arctic forest separating it from the lowest peat. Thus by his own admission there were ten strata, each indicating a climatic fluctuation, and his six floristic invasions to be

TABLE 1

BLYTT'S SCHEME FOR MIGRATION, NORWEGIAN FLORA, WITH
APPROPRIATE CLIMATES
(Bot. Centr. 7–8: 299 et. seq. 1881)

Present flora	Bog layers	Plant materials	Moisture	Temperature	Designation
6. SubAtlantic (Southernmost coast of Norway)	10. Root layer 9. Peat	Dry moors SubAtlantic flora. Beech? Spruce?	Drier Wet	Cooler than Atlantic Cooler than Atlantic	7. Present 6. SubAtlantic
5. Subboreal (S. Norway inland, low alt., up to 200 ft.)	8. Root layers and forest remnants	"Subboreal flora"	Dry	Cooling?	5. Subboreal
4. Atlantic (W. coast of Norway)	7. Peat	Stems and leaves of Quercus sessiliflora.	Wet	Warmer than now	4. Atlantic
3. Boreal (S. of 64th parallel; Inner fiords and mts. up to 2000 ft.)	6. Root layers, etc.	Linden, etc.? Hazel, oak, Bird cherry. Extensive deciduous forests	Dry	Warmer than now	3. Boreal
2. Subarctic (N. Norway Mts. of S. Norway)	5. Peat 4. Root layers, etc. 3. Peat	Pine stems Birch, aspen leaves	Wet Dry Wet	Cool? Cold-Subarct. Cold-Subarct.	2. Subarctic
1. Arctic (Present arctic lands only)	2. Clay 1. Last segment of Glacial time	Arctic plants Willow, birch	Dry Wet	Cold, arctic Glacial	1. Arctic

accounted for. Table 1 represents the reviewer's effort to condense Blytt's correlations. A certain awkwardness of fit is evident. This was in part eliminated by later simplifications, partly by Blytt, partly by his pupil and friend, Rutgers Sernander (68).

Study of Blytt's original essay of 1876 establishes its right to be considered a botanical classic. Its tone is restrained, inductive and tentative in dealing with the mass of varied evidence presented. American readers will be puzzled by the archaic use of "pine" for

Picea and "fir" for *Pinus*.   Blytt did not have available the superb record of changes in Baltic levels that was later worked out, nor did he have the benefit of our present knowledge of the Pleistocene. Yet he certainly anticipates most of the later criticisms raised by Gunnar Andersson and meets them fairly and reasonably.   At no time does he pretend to do more than formulate a theory.

How then could Blytt become vulnerable to the savage attacks of Andersson?   On this score his first paper gives two hints, confirmed by a reading of his subsequent papers.   While conceding the importance of local physiographic change, he tends *a priori* to subordinate its rôle to that of climate in solving his problem.   And he is obviously intrigued by the suggestive ideas of Croll (16) who saw the source of climatic oscillations in a mathematical variation of the earth's axis through long periods of time.   While these hypothetical periods were of far greater magnitude than his own, their supposed regularity appealed to him (12).   Doubtless this reenforced his later growing tendency to formalize his own theory of alternating wet and dry periods until, in the words of von Post (54), it became a straight-jacket for students of European climatic history.

III. THE PERIOD OF DISPUTE, 1881–1910

Whatever may be said of Blytt's logic or prudence, he had performed the genuine scientific service of formulating a statement definite enough to be tested.   His most aggressive and determined critic was Gunnar Andersson (5) ; his staunchest supporter, Rutgers Sernander (66, 67, 68).   The resultant debate is full of interest, human and scientific.   It presently enmeshed many great biologists and geologists of Europe, and in the end became intensely personal.

It seems appropriate, however, to outline the various trends of opinion which developed, rather than to trace in detail the entry of brief and counter-brief.   To the reviewer, the following several views represent the general outcome:

Disbelief in postglacial xerothermy.   Since xerothermy implies the conjunction of warmth and dryness, it is probably fair to say that Andersson's position (4) was one of disbelief (1893).   Studying bogs over a wide area, he confirmed the general findings of Steenstrup (69), except that spruce succeeded oak in the north, as did beech in southern Scandinavia.   By actual count Blytt's regular alternation of peat and forest mold was not found in all bogs (4),

although Blytt never claimed it would be. Andersson conceded a milder climate before and during postglacial submergence (Litorina sea) of the Baltic area, but considered it to be warm and moist, as evidenced by the abundance of mesophytic·deciduous trees. He emphasized, and rightly, the importance of local change.

Graebner (34) in 1910 rejected the use of relict species and communities as evidence of past climatic change. He considered that bog history was too much subject to local influence to be trustworthy proof of climatic changes. At the same time Ramann (55) considered the presence of a weathered layer (Grenz-Horizont) between the old and new Sphagnum in many high-moors not a reliable evidence of climatic dryness. Much weight had been attached to this, as will be seen; but Ramann thought this weathered layer often a product of local drainage changes. About the same time Brockmann-Jerosch (13), in characteristically brusque fashion, rejected the evidence for xerothermy. In 1912 (14) he went further and insisted that Nathorst's findings of arctic plants in glacial clays was no proof of glacial climate much different from the present. His basis was the presence of remains of more temperate vegetation with those of arctic plants.

An interesting skepticism was that of Krause (42) who did not deny the possibility of climatic effects but who suggested that Nehring's steppe conditions in Germany might have been due to local salinity which prevented growth of trees. This absence of trees might in his opinion produce local climatic effects. He called attention to the fact that even in Asia forest normally intervenes between tundra and steppe.

It should be understood that disbelief in postglacial xerothermy does not of necessity imply rejection of relicts as evidence. It may mean merely that the relicts are believed to have persisted from interglacial or even Tertiary times. Penck, for example (52), at first claimed the loess and steppe fossils to be interglacial. His views carried weight because of his detailed studies on glacial cycles in the Alps.

So far as this reviewer can determine, the skeptical opinions just outlined represent a minority. But their influence persists. Or perhaps it might be fairer to say that they represent a segment which refuses to be influenced by anything save overwhelming evidence.

Subsequently Penck and Brückner (52), Schulz (63) and

Olbricht (49) all emphasized the possible importance of interglacial, as well as postglacial, warm, dry climates.

Belief in early postglacial xerothermy. This position rests on three-fold evidence, already cited: the loess (57), the steppe animal fossils (47); and Mediterranean plants in the Alps (40). Kerner reiterated his views on relicts in the Alps in 1888 (41), Nehring his assignment of position to steppe fossils in 1890 (48).

In 1890 these views obtained strong support from Briquet (8) who at that time postulated one glaciation followed by a xerothermic period of steppe and loess formation. This in turn he believed to be followed by a moister, cooler "forest period". During this steppe period the steppe fauna came into mid-Europe, and the steppe-flora with it, while the Mediterranean flora moved northward. Briquet was a student of Alpine flora. Later (9) (1907) he asserted that only a postglacial xerothermic period can explain the reentrance of certain elements in the Swiss flora.

Certainly the idea of an early postglacial dry period was acceptable to many. It was consistent with the known pattern of off-glacial winds (*cf.* Tutkowski (72)), with rapid melting of the glacial ice, and with the supposition that loess was glacial flour, moved by dust-storms characteristic of dry climates.

Andersson himself accepted this idea but insisted that the early dry climate was cool. It preceded his warm maximum which was wet and earlier than the warm dry subboreal period of Blytt and others, whose existence Andersson refused to accept. Krause (43) by this time (1910) agreed with the view of Andersson and identified the early cool dry period with Briquet's xerothermic. Since Briquet was working about as far south of Andersson as New Orleans is south of Toronto, this was not so inconsistent as might appear at first glance.

For Germany, Werth (79) considers that Andersson's early warm maximum is substantiated, but not the late warm dry subboreal. This warm maximum he considers to have been marked by a Rhenish or French winter climate for most of Germany. He places it during the time of the Litorina Sea, 8000–4000 B.C., with a maximum about 7000 B.C.—considerably earlier than the date of climatic optimum established by de Geer. Since Werth's paper appeared in 1928, his judgment is of particular interest.

Belief in later postglacial xerothermy. One of the most confus-

ing features of the decades of polemic is the fact that purely relative terms, such as "cool", "warm", "dry" and "moist", were bandied about by men working over an area as large as the eastern United States and much more varied topographically. It is necessary also, in reading the literature, to bear in mind the relative character of indicators. Thus the appearance of beech, for example, might indicate increasing humidity in a region of oak, and the reverse in a region of oceanic peat. Oak in its turn might bear a similar relation to steppe on the one hand and beech on the other. Moreover, the oaks themselves vary in climatic preference.

Blytt's original theory appears to have included two relatively warm, dry periods, the boreal and subboreal, separated by his warm moist "Atlantic". Either or both might have been properly called "xerothermic". It is of interest to note, however, that his so-called subboreal group of plants (11) in Norway actually includes the more xeric steppe flora, among others the Russian thistle, *Salsola kali,* which is generally such a dependable indicator of semi-arid high plains climate in America.

Meanwhile Andersson (4) had examined 121 Scandinavian bogs for evidence of the precise, systematic alternation of peat and forest mold claimed by Blytt. Of the 121, 77 failed to show the expected agreement. In Germany, Weber (1893) began his own intensive studies of peat layers, using not only gross evidence but studying microfossils, notably pollen (75). The outcome of his studies was the description of a weathered layer "Grenz-Torf" in the upper half of many moors (76). This consisted of humus between the so-called old and new *Sphagnum* layers. It was called the "Grenz-Horizont" and was believed by Weber to indicate the existence of a late postglacial xerothermic period, too warm and dry for peat formation. Its position seemed appropriate to the subboreal as christened by Blytt.

Rutgers Sernander had been a student of Blytt and was impressed by his theory. Accepting its main outlines, he (68) simplified it considerably. In particular he emphasized the importance of the subboreal as the period of most marked warm, dry climate. To this position, the "Grenz-Horizont" theory gave powerful support. Weber (77) for his part endorsed the xerothermic subboreal and the subsequent cooler moister subatlantic. But for the earlier part of postglacial time he refused to follow Blytt and Sernander, agreeing instead with Andersson.

Later studies of the Grenz-Horizont (*cf.* Gross (37)) suggest that, like Blytt's peat and forest mold layers, it may not be so universal as thought, and may be also the product of strictly local drainage changes.   Yet these criticisms do not completely discredit its significance when supported by other types of evidence.

Belief in more than one xerothermic period.   While the emphasis of both Sernander and Weber is on the late postglacial warm dry maximum, it is possible that they should not be excluded from this fourth grouping of opinion.   Blytt himself clearly belongs here, as well as a number of more objective students, for Blytt's emphasis was on the recurrence of dry periods, from the beginning of the Tertiary on.

Penck and Brückner (52), in their studies of Alpine glaciation, demonstrated a history of repeated advance and retreat, presumably involving changes in humidity as well as temperature.   Schulz (62), following the procedure of Forbes and Blytt, recognized four climatic groups in the flora of Germany, inferring their entrance variously during eight periods, including the last glacial.   He was a persistent critic of Briquet who, in his opinion had lumped into one (early) xerothermic period the effects of more than one, plus those of an interglacial warm dry climate.   It should be noted that Briquet (9) presently conceded the existence of a loess earlier than his xerothermic.   Turning his attention to Sweden, Schulz (61) insisted that Andersson's ideas did not square with those of Penck and Brückner's Alpine climatic cycles.   However, the extent to which these cycles could serve as a norm for all of Europe was itself questioned.

Others who believed the evidence to indicate more than one period of continental climate include:

Olbricht (49) who, finding four sets of terraces on Lüneberger Heath, considered them to represent alternating periods of much and little moisture.

Wahnschaffe (74), tracing the changes in Baltic levels and correlating them with climatic changes and fossils.   He believed that loess and steppe fossils might well have been glacial, and places the Grenz-Torf at the end of the Litorina sea, whose position in the time scale will be shown presently.

Stoller (70) presented a similar sequence, announcing a brief continental climate for northern Germany during the period of the arctic

Yoldia sea, with steppes and dunes in the South. The later Ancylus lake and early Litorina sea period were also continental—warm and dry. This he believed marked the entrance of oak and formation of the Grenz-Horizont, which Wahnschaffe had placed later. The subsequent entrance of beech and alder marked, according to Stoller, a climate still warm but moist.

The preceding paragraphs do not, by any means, include the names and views of all workers. They do, however, give a fair sampling of the divergence. For further details the extensive original sources must be consulted. A valuable bibliography has been prepared by Antevs (7), while important abstracts and discussion are to be found in Clements (15). The climax of the controversy was reached during the 11th International Geological Congress at Stockholm, whose publications include a special volume on postglacial climate (68).

IV. THE PERIOD OF CRITICAL REEXAMINATION, 1910–

The field of discussion was by now a mêlée rather than a duel of champions, although most participants were roughly aligned with either Andersson or Sernander. Advocates of no xerothermy found Andersson's ideas more congenial, while those who believed in more than one period were obliged to agree in part at least with Sernander. The contrasting views were as follows: (66)

Sernander	Andersson
SubAtlantic Cooler, moister (Climatic Deterioration)	Climatic deterioration Cooler and wetter
Subboreal Warm Dry (The Xerothermic Period)	
Atlantic Warm, moist (Climatic Optimum)	Atlantic Warm, wet Climatic Optimum (Neolithic)
Boreal Cool, dry (Continental Transition)	Boreal Very dry, ultimately warm Only dry part of P.G.
Late Glacial, Early Postglacial Cold Arctic and SubArctic	Late Glacial, Early P.G. Cold

It will be noted that Sernander's views are considerably simpler than Blytt's original scheme. Because of the frequent appearance

in literature of the term "Blytt-Sernander hypothesis", it is of interest to record that this designation was coined by its critic, Andersson (8), and deeply resented by Sernander (67). The latter contended that, with all respect for Blytt, he had approached the problem in an independent spirit and developed his own statement in accord with his newer findings.

It is fair to say that the whole problem had its origin in the attempt to explain certain floristic paradoxes. These may be reviewed: (a) Forbes' recognition of constituents in the British flora having diverse geographic origins, (b) Gray's finding of species common to Japan and eastern North America, (c) Blytt's report of six diverse groups in Norway, (d) Kerner's finding of Mediterranean plants in the Alps, (e) Schulz's distinction of five floral elements in Germany, (f) Briquet's xerotherm members of the Swiss flora. On the basis of any theory whatever, such facts call for a rational explanation. Even the Special Creationists conceded this.

Once launched, and with the acceptance of continental glaciation as an event of reference, inquiry turned to recent stratified organic sediments, *i.e.,* peat and associated deposits. So far as gross plant remains are concerned, the evidence for Scandinavia was remarkably consistent. In the clay bottom were Arctic plants as found by Nathorst, *viz: Dryas,* dwarf willow and birch; in the peat above that, in ascending order, pine and birch; then oak, hazel and an increasing number of deciduous trees; then beech in the south, spruce farther north. Finally at the top, conditions were equivocal; in places the forest clearly overgrown with peat, suggesting accumulating moisture; in other places humus, topographic factors and human activity had affected surface change.

Whatever else may be said of the peat column, it is the sort of sequence to be expected if postglacial climate had gradually moderated to a condition favorable for oak, then "deteriorated".

The peat column was also scrutinized for evidence from its physical texture, less successfully. Alternating layers of peat and forest mold or stumps, suggesting, respectively, wet and dry climate, did not reveal the regularity of pattern needed to convince skeptics. The presence of the Grenz-Torf, or Grenz-Horizont, a weathered layer formed subsequent to the entrance of deciduous forest elements, and taken to indicate a warm, dry period, was most gener-

ally accepted. But here again the prevalence of variation and the undoubted effect of local changes gave the skeptics room for honest doubt. Yet to tell the truth, the operation of a generally dry climate should be subject to local modification, and interruptions in the Grenz-Torf ought to be expected even by its advocates. As is true of surface conditions, then, this evidence is equivocal.

Such in brief is a résumé of the problem developed by botanical study and discussion during the nineteenth century. To these facts should be added the evidence from vertebrate fossils in Germany where tundra animals were followed by steppe animals and these in turn by forest fauna. Since the Grenz-Torf—if authentic—came after the entrance of forest, it can not correspond to the period of steppe animals. But these might well have been present in Germany while tundra was being followed by pine-birch forests in Scandinavia, if not earlier.

It is now in order to review evidence, much of it critical, from other sources. These are as follows:

Changes in level in the Baltic region. In tracing these changes and their concomitant events, molluscan remains have been of prime importance. As early as 1864, Sars (59) had demonstrated the former presence in the Scandinavian area of the mollusc, *Yoldia arctica,* whose species name is descriptive. Subsequently the following sequence was worked out (50):

The Present Baltic Sea—relatively brackish—too much so for oysters except near the mouth. Now characterized by the clam *Mya,* earlier by the snail *Limnaea borealis.*
The Litorina Sea—warm, salty—characterized by the marine snail *Litorina* and by oysters whose shells abound in middens.
The Ancylus Lake—freshwater—marked by the snail *Ancylus fluviatilis.*
The Yoldia Sea—cold—showing *Yoldia,* as above noted.
An Icefront Lake—freshwater.

Here is a standard scheme of reference for postglacial history. That such changes would have an effect on local climate is obvious. That in general the fauna indicates major climatic change seems equally clear. The temperature climax suggested by the warm Litorina Sea is especially striking.

Glacial advance and recession. The earliest efforts to account for glacial and postglacial phenomena were influenced by the Doc-

trine of Special Creation.   The former was designated "Diluvial", the latter "Pluvial".   These terms still appear occasionally in the literature, although abandoned by Lyell and others early in the 19th century.

There followed an attempt to explain continental glaciation as due to a widespread arctic sea with floating ice-bergs.   This view was held by Forbes (26).   Slowly the view of Agassiz regarding continental ice-sheets prevailed, but for a long time the work of the Pleistocene ice was considered to represent a single prolonged invasion.   Later the presence of several successive drift-sheets was recognized, each recording a major advance and retreat of the ice. The ice-age was accordingly divided into glacial epochs, separated by interglacial intervals.   With greater refinement, minor advances and retreats of the last great ice-sheet have been recognized.   This has afforded a strictly inductive basis for the theory of climatic fluctuation.   At least one interglacial has been proved to be warmer than the present.   Clearly the whole xerothermic discussion involves the question as to whether the postglacial has been warmer (and drier) than the present.   If so, its pattern is suggestive of an interval between ice advances, rather than a resumption of the comparative uniformity of climate which has marked most of geological time.

Absolute agreement on the number and character of glacial movements has not been attained.   The following, however, may be taken as a representative view for Europe:

The present and recent past
A warmer period
The Daun readvance of Alpine glaciers (cool)
A warm period
The Gschnitz readvance in Alps
A warm period
The Bühl readvance in Alps
The Würm—last major glaciation— interrupted warmer Achen interstage, a period of retreat.

The Riss-Würm Interglacial
The Riss—third major glaciation
The Mindel-Riss Interglacial—prolonged
The Mindel—Second Major Glaciation
The Gunz-Mindel Interglacial— doubted by some.
The Gunz—First major glaciation.

Whatever the future may reveal as to the details of this schema, it clearly affords ample justification to those who seek to explain present floristic patterns on the basis of migrations influenced by past climatic fluctuations.

Human cultural remains.   The type of culture and its position in time and space may afford important clues to past conditions.

The presence of Pleistocene man in the caves of southern Europe, with arts closely akin to those of the modern Eskimo, is well established. The northward movement of climate and vegetation in postglacial time is reflected in successive human use of reindeer, moose and stag in western Europe (51).

There is some agreement that the neolithic arts of agriculture and polished stone, which had their origins in Asia, were favored by open steppe or park-like country (47). Thus periods of drier climate would tend to encourage advance of this type of culture into northern and western Europe. Moister forest conditions would tend to check it in favor of hunting cultures. This distinction should become less marked with advance of metal working and use of draft animals which would make easier the tasks of drainage and of removing forest and holding it in check. Likewise, in forested regions, use of fire for clearing would depend largely upon the degree of dryness.

As will be seen, the cultural history of Scandinavia has now been dated with great precision, and there is increasing certainty for the remainder of Europe. In Scandinavia the earlier neolithic accompanied the pine and birch period—the boreal of Sernander. Later neolithic and bronze corresponded to the oak, while expansion of beech in western Europe is known to have occurred during and since Roman times. The question is, whether after the warm moist Atlantic Period, following the pine and birch (and hazel), and introducing deciduous forest, there was again a drier period, the subboreal, that may have favored the undoubtedly high bronze culture in Scandinavia. Such a period would correspond to the Grenz-Torf in Germany, if there is one, and would be a late, dry, warm, subboreal xerothermic.

Much more difficulty is encountered on the continent. Krause (43), for example, denies the possibility of any climatic change in later postglacial times. Changes in vegetation since the end of the Ancylus Lake when conifers were replaced by deciduous trees he attributes to normal succession and human interference. He places the older neolithic during Andersson's transition from *Dryas* to birch, which he concedes to be dry, but subglacial and only relatively warm. This, he holds, represents the "xerothermic" period of Briquet and in Germany. Gradmann (33) affords an instance of opposing views, in which a later, neolithic, warm, dry climate,

characterized by steppe conditions, Grenz-Torf, and loess, is postulated.

Annual varves in clay of Ice-Front lakes. The Swedish geologist, de Geer (20), has employed the annual laminations in glacial lake sediments to establish a postglacial time-table. The light broad bands of clay due to summer melting are separated by narrow dark bands. Breadth of summer bands fluctuates with character of the season, and thus deposits in different lakes can be connected. Since successive positions of the ice-edge are indicated by surface features, this dating can be applied to stages of retreat and pause.

By de Geer's chronology, the date of maximum extension of the last major, or Würm, glaciation was about 20,000 B.C. This, it will be recalled, extended to the Alps and Carpathians but did not cover the Low Countries of western Europe.

There followed a period of 11,000 years, during which the ice retreated to what is known as the Fennoscandian moraine, exposing the southern portion of Scandinavia, surrounded by the Yoldia arctic sea. During this time the retreating ice was bordered by arctic vegetation. Between 9000 B.C. and 7000 B.C. was another rapid retreat, to the halt known as the Ragunda pause. This was marked by the Ancylus Lake and an ice-border of temperate vegetation.

By 6500 B.C., the " official end of the Ice Age", the lobe had split. 4000 B.C. marked the initiation of the warm salty Litorina Sea, with the "Climatic Optimum" of warmth and moisture between 4000 and 2000 B.C. This, according to de Geer and Montelius, saw the beginning of the Neolithic in Scandinavia, with the first appearance of bronze toward the end of the climatic optimum, about 2200–1700 B.C. (51). This last event corresponds in position with the supposed subboreal which may well have been drier, since it was accompanied by an elevation of land surface.

The length of time during which Germany was ice-free as compared with Scandinavia explains clearly enough why the comparatively neat sequence worked out for the latter area cannot be rigidly adapted to the continent. Whatever may have been the effect of any post-Atlantic (*i.e.,* Subboreal, post-Climatic-Optimum) desiccation on the continent, there was ample time for an earlier dry steppe-period, following the tundra, as supposed by numerous workers. Moreover, according to Osborn (51), neolithic culture

had its Asiatic beginnings in 18,000 B.C., was present in Persia in 16,000 B.C., and had entered the Mediterranean by 12,000 B.C.

There is thus no *a priori* reason to assume that a neolithic steppe-culture might not have expanded into central and northwestern Europe on the heels of an appropriate climatic fluctuation, long before the supposed warm dry subboreal and the associated Grenztorf of later postglacial time.

Soil profiles. The contrast between forest podsols, with relatively thin humus layer, and the deeper black subhumid steppe soils is well known. Where former soils have been preserved *in situ,* their evidence as to vegetation and climate is important. In view of this fact, the work of Jonas (39) and Altehage and Jonas (3) casts light upon the problem just discussed.

These authors report that German bogs show an unbroken record of forest back into late-glacial time. Below the forest layers, however, are black soils (Trockenrasenboden) indicative of dry grass tundra or steppe. This accords with the findings of Nehring (47) who placed the steppe between tundra and forest. It is also in harmony with orthodox geological opinion which associates aridity and loess formation closely with periods of glacial retreat.

Pollen statistics. In 1910 Andersson (5) paid rather grudging respects to one scientist whom he considered a follower of Sernander. This was L. von Post who was then undertaking to study the microfossils in peat. Andersson admitted that this enterprise might be hopeful, but not too hopeful. Weber (75) in 1893 had made extensive use of pollen in attempting to correlate various peat layers, and Stoller in 1910 had also found it useful (70).

von Post began to study the pollen statistically, and by 1916 had developed (53) the method now known as pollen statistics or pollen analysis. By this means the relative abundance of various kinds of subfossil pollen in peat could be used to indicate vegetation other than that growing immediately in the bog. Success of the method rests upon the fact that most commoner forest trees are anemophilous, and that most kinds of pollen are remarkably well preserved under favorable conditions.

Pollen statistics have proved themselves a powerful tool for research, but are not, of course, foolproof. The literature has become enormous, and thanks to Erdtman, is well catalogued (22). American readers interested in the subject are referred to an earlier re-

view by the present writer (64), a chapter by Erdtman in Wode-house's book on "Pollen Grains" (23), a forthcoming book by Erdtman (24), and in particular to two excellent articles by Godwin (31, 32). In general, the results of pollen statistics confirm the re-sults already outlined in this paper. There is one important excep-tion. So far as this reviewer is aware, they give no evidence as yet on the vexed problem of a steppe climate preceding the forest period in continental Europe. This is not strange, since a grassland climate is not generally favorable to peat formation and pollen pre-servation. There are exceptions, as in the case of Lane's studies (44) of interglacial peat in Iowa, and the writer's analysis of canyon silts from Arizona (65).

Pollen statistics are most useful when conducted with a full under-standing of numerous other sources of evidence. These include local physiographic history, plant succession, human and other biotic factors, sedimentation and floristics. It has already served to emphasize the diversity of conditions within the continent of Europe, and to act as a brake upon sweeping generalizations re-garding synchronous climatic changes.

This is well, for the old cataclysmic idea dies hard. No doubt of it, much of the bitter difference of opinion has come from inferring, and expecting to find evidence of changes more sweeping and abrupt than have really occurred. In the operation of climatic change upon plant migration, Asa Gray's words regarding the puzzle of the eastern prairies apply: "there must be a debatable border where slight causes will turn the scale either way". The study of climatic changes seems to the reviewer to be a matter of tracing the slow shifting of such debatable borders through the cen-turies.

In Poland, Szafer (71) has utilized pollen statistics to map the moving ranges of spruce and beech by a device which he calls iso-pollen lines or contours. These clearly suggest continentalization of climate before and after, but not during, the period of maximum warmth. The existence of such a time of greatest warmth is now generally conceded, even by Andersson, whose studies of the north-ward extension of hazel reveal it. Szafer's maps show it by the northward extension and subsequent retreat of beech.

More than that, Szafer clearly shows that *Picea* had survived glaciation, not only in Carpathian and Russian refuges, but as scat-

tered disjuncts in western Europe. More recently Du Rietz (21) has added similar complications with evidence that elements of both the British and Norwegian floras had persisted locally throughout glaciation. Geologists are inclined to be skeptical, as many of them are towards Fernald's similar views regarding glaciated North America (25). But in both instances, the floristic facts are there, to be explained.

Climatology. From this exceedingly technical and rapidly growing field it will suffice to note that no *a priori* reason exists to doubt the probability of significant climatic fluctuations during postglacial time. As Abbot (1) has indicated, change is the rule, not the exception. Variations in solar radiation and in ocean currents are known to occur, with measurable consequences. Among the most significant of these consequences are the effects upon organic life; and climatologists, for all their astronomical preoccupation, are hopefully attentive to all evidence produced by students of plants and animals.

V. CURRENT JUDGMENT

The floristic problem which had originally concerned Blytt now appears in a broader setting, as part of the whole question of postglacial movements of vegetation from those localities to which it had been driven by the advancing influence of the ice. Such movements were influenced by events far beyond the modest limits of Norway, and by changes in the pattern of land and water in Scandinavia itself. We may, with advantage, quote from Godwin's comment (32) on the Blytt-Sernander hypothesis:

"With greater or less success this climatic schema has been extended outwards from Sweden to other parts of Europe, and though often yielding a consistent picture of climatic and vegetational history, it does not apply unmodified to the whole of Europe, nor is it adequate for the complexity of climatic history now recorded in Sweden itself. Von Post has, therefor, proposed a basic threefold division of post-Glacial time which is applicable to the whole of Europe and which corresponds to the main features of forest development:

'1. The stage of the approach to the warm period, characterised by the appearance and first increase of relatively heat-loving trees of different kinds.

'2. The stage of culmination of these forest elements.

'3. The stage of the decrease of the characteristic trees of the warm period and the appearance or the return of the dominant constituents of the present day'.

"The use of such a schema for Europe as a whole has clear advantages over the Blytt-Sernander schema, and it has already been adopted by some workers, notably Keller. *It is perhaps an advantage that this schema avoids one of the outstanding problems of post-Glacial climatology, namely the possibility of a secular dry period corresponding to the sub-Boreal of Blytt and Sernander*". [Italics, the reviewer's]

To Godwin's considered opinion we may add the judgment of Hesselman (38), expressed in his account of the life and work of Gunnar Andersson. The bitter conflict with Sernander, in his opinion, has been brought to a Solomonic verdict. Through refined techniques—chiefly pollen analysis—evidence has accumulated to support the existence of a recent, warm, dry, subboreal. But Hesselman also notes that much evidence has accrued to support Andersson's idea that the warmer part of postglacial time occurred considerably earlier than the subboreal.

This shrewd estimate of the situation leaves untouched the question of an earlier dry boreal in Scandinavia, its intensity, and its position in time relative to the supposed steppe-period in Germany.

In view not only of the existing paralysis of scientific work and communication but of the divergence of opinion among Europeans who have examined firsthand evidence, it would be presumptuous for this reviewer to make any final estimate. But he is convinced that warm continental organisms have had more than one opportunity to expand beyond their most characteristic present confines. Such migration may have taken place during the Tertiary, during one or more interglacial periods, during the supposedly warm and arid conditions of rapid ice retreat, or most recently during the subboreal, Grenz-Torf, period, if it existed. So far as glacial retreat is concerned, the pattern of moraines clearly shows that it was not a continuous affair. There were times of pause, and even readvance during the last great melting.

Under these circumstances, to select a single time and invest it with sole responsibility for xerothermic effects would demand overwhelming proof. Such proof would have to establish, among other

things, the fact that the relatively moister and cooler periods of climate wiped out any traces of warm, continental vegetation which might have made their way previously into northern and western Europe. Considering the known capacity of any but the most uniform landscape to diversify the impact of a given climate, this would seem to be a large order.

VI. XEROTHERMIC THEORY IN NORTH AMERICA

This topic requires a separate review. Meanwhile, the reader should consult Gleason's "History of the Development of Vegetation in the Middle West" (29), also a previous review by this writer (64). As in Europe, so in North America are there numerous instances of warm and continental plants beyond their usual limits. Most striking, however, were the islands of prairie in the deciduous forest region east of the prairies proper. In other words, there is a definite problem of disjunct and supposedly relict *communities* as well as of species.

Much critical work was necessary to establish as a fact what many had surmised—that the distinction between grassland and forest was based primarily upon a difference in available moisture. With that clearly demonstrated, the likelihood of an earlier, more continental climate than the present, during which prairies came farther east than now, was certainly increased.

Today, while the evidence is complex and not wholly consistent, we have every reason to believe that for North America more than one opportunity has existed for northeastward movement of vegetation and floristic boundaries. In the reviewer's opinion, the problem is no longer to be: "Was there a xerothermic period?". Instead, the proper question must be for North America, as for Europe: "What have been the relative positions in time, the relative intensities, and the residual effects of the several—Tertiary, interglacial, and postglacial—periods favorable to xeric, thermic, and to xerothermic expansion?"

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